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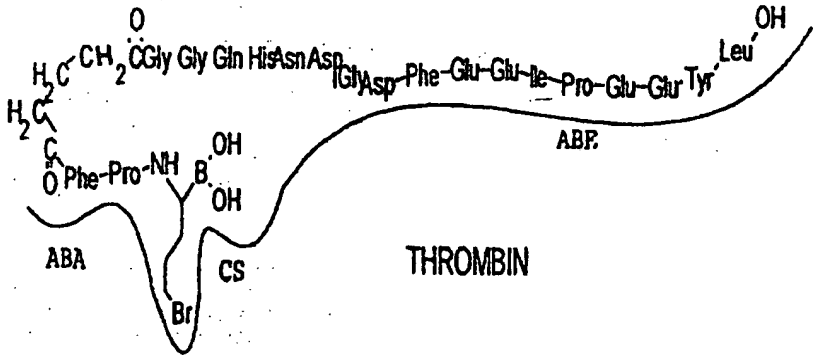

Key

ABA = apolar binding area

CS = catalytic site

ABE = anion binding exosite

(57) Abstract

Bifunctional peptide thrombin inhibitors and methods of their manufacture are provided. The inhibitors have an anion-binding exosite associating moiety joined to a catalytic site directed moiety. A spacer peptide and a non-peptide linker moiety enable both the anion-binding exosite associating moiety and the catalytic site directed moiety to bind simultaneously to a thrombin molecule thereby permitting the treatment of thrombosis.

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THROMBIN INHIBITORS

This invention relates to thrombin inhibitors and thrombin substrates. and to their use for example in the treatment or prevention of thrombosis.

Cardiovascular disease is a major cause of mortality, with incidence across the world higher than that of cancer. Acute events in the disease state. such as myocardial infarction. stroke. peripheral arterial occlusion and venous thromboembolic disease have recently been understood to be precipitated by formation of thromboembolic clots. This clot formation. as well as the aetiology of the disease state. e.g. formation of atheromatous plaque. has been shown to be mediated by the coagulation serine protease enzymes which control also the normal haemostatic balance of the blood. Modulation of any one coagulation protease. especially thrombin, has been shown to control thrombogenesis. This has led to the development of inhibitors of thrombin to prevent thrombotic events in the clinic.

Thrombin cleaves peptide bonds by a mechanism involving the catalytic triad of Asp-His-Ser residues in the active site of the enzyme. Thrombin inhibitors have been designed which use functional groups. e.g. CO-H, $B(OH)_2$. $P(O)(OR)_2$, beta lactam, chloromethylketone. to interact with the triad and thereby block activation of the substrates.

Inhibitors selective for thrombin have been prepared by making peptide inhibitors comprising peptide sequences that bind preferentially to subsites unique in the thrombin. Typically these sequences mimic the structure around the scissile bond of the natural substrate of thrombin, which is fibrinogen. For example, selective peptide inhibitors of thrombin typically incorporate a sequence based on Phe-Pro, or more generally (aa)-Pro, where (aa) is some hydrophobic amino acid or analogue thereof.

The amino acid residue which provides the carbonyl group of the scissile bond of a peptide sequence is designated "P1". Successive amino acid residues on the N-terminal

side of residue P1 are designated P2, P3, P4 ... etc; amino acid residues on the C-terminal side of residue P1 are designated P1', P2', P3' ... . In fibrinogen, P1' is glycine and P2' is proline. The protease contains a "specificity pocket" which recognises the side chain of the P1 amino acid. Thrombin normally recognises P1 residues with arginine-like or serine-like side chains.

Peptide inhibitors of thrombin have been made in which the P1 terminal carboxy group is replaced by another acid group, e.g. a boronic acid group or a phosphorus oxyacid function. The P1 terminal carboxy or heteroatom analogue group may be derivatised, for example to form an ester, an alcohol, a thiol or an amine or to replace the OH groups of boronic acid with fluorine. The identity of the derivative moiety is not critical and may be selected according to the desired use of the target compound. Peptide inhibitors having a boron or phosphorus heteroatom analogue group at the P1 residue are described in, for example, WO 92/07869 (equivalent to USSN 08/317,837), EP 0471651 (which corresponds to US 5288707) and USSN 08/240,606, the disclosures of which are incorporated herein by reference. Inhibitors having a P1 sulphonic acid group and derivatives thereof are described in Wong, S.C., Green, G.D.J., and Shaw, E., *J.Med.Chem.*, 1978, 21, 456-459, 'Inactivation of trypsin-like serine proteases by sulfonylation. Variation of the positively charged group and inhibitor length'. As examples of such peptide thrombin inhibitors, it may be mentioned that α-amino boronic acid peptides have been prepared because of the favourable binding energy of the interaction of boron with a nucleophile, such as the lone pair of the Ser hydroxyl or His imidazole group, to give a tetrahedral boronate intermediate which mimics the shape of the "transition state" formed during substrate cleavage and so is tightly bound to the enzyme. The α-amino group of such α-amino boronic acid compounds forms the P1-P2 amide link of the peptide.

The literature teaches that thrombin is strongly inhibited by α-aminoboronic acid-containing peptides: Tapparelli, C.; Metternich, R.; Erhardt, C.; Zurini, M.; Claeson, G. Scully; M.F.; Stone, S.R. "In Vitro and In Vivo Characterisation of a Neutral Boron-containing Thrombin Inhibitor" *J.Biol.Chem.* 1993, 268, 4734-4741; 'Boroarginine

Thrombin Inhibitors' Kettner, C., Mersinger, L.. & Knabb, R. (1990) *J.Biol.Chem.* 265, 18289-18297;. Taparelli, C.; Metternich, R.; Erhardt, C.; Cook, N.S. "Synthetic Low-Molecular Weight Thrombin Inhibitors: Molecular Design and Pharmacological Profile" *Trends Pharm.Sci.* 1993. 14. 366-376.

EP 0118280 and equivalent US patents US 4638047 and 4772686 describe peptide thrombin inhibitors comprising amino acid residues on the C-terminal side of the scissile bond in which the P1-P1' scissile peptide bond is replaced by a non-hydrolysable isosteric linkage, namely $-COCH_2-$, $-CHOHCH_2-$ or CH_2NH-.

The state of the art, therefore, includes peptide thrombin inhibitors. It will be understood that the term "peptide" includes peptide analogues. Such inhibitors are known in which there is at the carboxy position of the P1 residue an optionally derivatised carboxy group or an optionally derivatised heteroatom analogue of a carboxy group, for example.

In terms of chemical structure, it may be said that the prior art comprises compounds included within the formula

$$X\text{-}(aa^4)_m\text{-}(aa^3)_n\text{-}(aa^2)\text{-}(aa^1)\text{-}Z, \quad I$$

wherein aa^1, aa^2, and aa^3 represent natural or unnatural acid residues and $(aa^4)_m$ one or more optional amino acid residues linked to the amino group of aa^3. Alternatively any one or more aa groups may be analogues of amino acid residues in which the α-hydrogen is replaced by a substituent. The sequence of amino acids and/or amino acid analogues binds to the thrombin active site. Suitable sequences are described later in this specification. X represents H or a substituent on the N-terminal amino group. Z is -COOH or a C-terminal extension group (carboxy replacement group), for example as known in the art. In preferred compounds Z is a heteroatom acid group, e.g. $-B(OH)_2$, $-P(OH)_2$ or $PO(OH)_2$, or a derivative thereof, for example a carboxylic acid ester, a dioxo-boronate [$-B(Osubstituent)_2$] or a phosphate [$-PO(Osubstituent)_2$] or BF_2.

Preferred heteroatom analogue groups are $-B(OH)_2$ and $-P(O)(OH)_2$; a less preferred heteroatom analogue group is $S(O)_2OH$. Amongst other possible Z groups there may be mentioned -CN, $-COCH_2Cl$ and $-COCH_2F$.

Derivatives of the acid groups include those in which inert organic groups, typically containing no more than 20 carbon and hetero-atoms, replace the hydrogen of any acid -OH group; the inert organic groups may be joined to the acid group through the intermediary of a functional group, such as carbonyl or amino, for example. In other derivatives, an -OH group is replaced by a substituent which may, for example, be an inert organic group or halogen, notably fluorine. It is also known to make compounds in which the acid -OH groups are replaced by -SH groups, which may be substituted, or amine groups. Representative inert organic substituents are hydrocarbyl and hydrocarbyl substituted by halogen or -OH; the hydrocarbyl moiety may contain an ether or ester linkage, for example.

The prior art may also be stated to comprise compounds included within the formula II:

W-A-Z II

wherein A is a group selected to have affinity for the specificity pocket of thrombin, W is a moiety selected to have affinity for the thrombin binding subsite and Z is -COOH or a replacement group therefor.

International application No PCT/GB96/00352 (Deadman et al.) describe novel peptide serine protease inhibitors in which the P1-P2 natural peptide linkage is replaced by another linking moiety.

Deadman et al provide in particular compounds of the formula III:

$$X\text{-}(aa^4)_m\text{-}(aa^3)_n\text{-}(aa^2)\text{-}\psi\text{-}(aa^1)\text{-}Z \qquad \text{III.}$$

wherein ψ is a linker between the aa^1 and aa^2 residues other than a natural peptide (-CONH-) group. and compounds of the formula IV:

W-ψ -A-Z IV

wherein, *inter alia*: A is a group selected to have affinity for the specificity pocket of thrombin. W is a moiety selected to have affinity for the thrombin binding subsite, ψ is a linker between W and A other than a natural peptide group and Z is -COOH or a replacement group therefor.

The most potent thrombin inhibitor known is hirudin, a family of isoproteins isolated from the glandular secretion of the leech Hirudo medicinalis. This single chain protein containing 65 residues has a high affinity for α-thrombin ($K_D 10^{-12}$ to 10^{-15} M) and does not inhibit other haemocoagulant enzymes. It consists of an active site blocking moiety, (Hirudin $^{1-48}$), a fibrinogen-recognition exo-site binding moiety, (often regarded as Hirudin $^{55-65}$), and a linker (often regarded as Hirudin $^{49-54}$), connecting these inhibitor moieties.

The binding between hirudin and thrombin is a two-step process. Initially, hirudin binds to a low affinity site on thrombin which is separate from the catalytic site. This binding involves interaction of a domain from the C-terminus of hirudin with an "anion-binding exosite" (ABE) in thrombin. Following the low affinity binding, the hirudin-thrombin complex undergoes a conformational change and the hirudin then binds to a high affinity site on the thrombin. This latter site corresponds to the active site of thrombin.

US 5196404 of Maraganore et al and corresponding international application No WO 91/02750 disclose bifunctional peptide thrombin inhibitors based upon the structure of hirudin. These bifunctional peptides are named "hirulogs". The hirulogs are described as mimicking the action of hirudin by binding to both the low affinity ABE and the catalytic site of α-thrombin. They contain a catalytic site directed moiety ("CSDM")

joined by a linker region to an anion exosite associating moiety ("ABEAM"). The CSDM is an amino acid sequence joined at its C-terminus to the linker.

The CSDM, which forms the N-terminus of a hirulog molecule, is a low molecular weight peptide which most typically is of the structure H-D-Phe-Pro-Arg-Pro. Peptides containing the P3-P2-P1 sequence D-Phe-Pro-Arg were already known in the art to be thrombin-specific.

The CSDM of a hirulog is joined at its C-terminus to a linker of suitable length (18Å-42Å) to enable the CSDM to bind to the catalytic site and the ABEAM to the anion-binding exosite. In a preferred compound (Hirulog 8) the linker is a peptide having the amino acid sequence $(Gly)_4$ and is linked to an ABEAM comprising amino acid residues 53-64 of hirudin. Hirulog 8 therefore has the sequence:

$$\underbrace{\text{H-D-Phe-Pro-Arg-Pro}}_{\text{CSDM}}\text{-}\underbrace{(\text{Gly})_4}_{\text{linker}}\text{-}\underbrace{\text{Hir}^{53\text{-}64}}_{\text{ABEAM}}$$

In general, the ABEAM may comprise the ABE binding domain of any molecule known to bind the anion binding site of thrombin.

The present invention provides novel bifunctional peptide thrombin inhibitors, in which the ABEAM does not form a C-terminal extension of the CSDM but preferably is joined to the CSDM at its N-terminal amino acid. In preferred embodiments the ABEAM is linked to the CSDM through a spacer peptide and a non-peptide linker moiety.

The CSDM functionally comprises a moiety of any of formula I, II, III and IV but is preferably such a moiety in which Z is a heteroatom acid group or a derivative thereof. The ABEAM may be the ABE binding domain of any molecule known to bind to the anion binding site of thrombin and, for example, may comprise any ABEAM mentioned in US 5196404 or WO 91/02750, or an exosite-binding analogue thereof. The two

binding domains are connected by a connector moiety which enables both groups to bind simultaneously to a thrombin molecule.

In another aspect the invention provides a method for preparing compounds of the invention by solid phase synthesis.

As used herein, "natural" amino acid means an L-amino acid (or a residue thereof) selected from the group consisting of:

Ala = alanine
Arg = arginine
Asn = asparagine
Asp = aspartic acid
Cys = cysteine
Gln = glutamine
Glu = glutamic acid
Gly = glycine
His = histidine
Ileu = isoleucine
Leu = leucine
Lys = lysine
Met = methionine
Phe = phenylalanine
Pro = proline
Ser = serine
Thr = threonine
Trp = tryptophan
Tyr = tyrosine
Val = valine

By "unnatural" amino acid is meant any α-amino acid (or residue thereof) other than the natural amino acids listed above. Unnatural amino acids therefore include the D-

isomers of the natural L-amino acids. Examples of unnatural amino acids include for instance: D-Phe, norleucine, hydroxyproline, α-carboxyglutamic acid, pyroglutamic acid, and other amino acids having side chain protecting groups and which are capable of incorporation into the peptides of the invention.

Where prefixed by "D" or "L", the foregoing names or abbreviations indicate an amino acid of D- or L-configuration respectively. A "D.L-" prefix indicates a racemic mixture of amino acids of the two configurations. Where no prefix is included, this means that the amino acid can be of either the D- or the L-configuration, except in the examples where residues are of L-configuration unless otherwise stated. For those groups of unspecified configuration in the text which can be of D or L configuration, L configuration is preferred.

Abbreviations and terms prefixed by "boro" indicate amino acids wherein the terminal carboxyl group $-CO_2H$ has been replaced by a group Z which is a boron functionality.

The term "analogue" when used in reference to amino acid residues or other moieties denotes an alternative to another group without implying that analogous groups impart the same properties to a compound. To the contrary, biological properties of compounds can be significantly changed by replacing a moiety with an analogue thereof.

Where asymmetric centres in formulae herein are marked with an asterisk (*), this denotes a stereo configuration of either D or L.

Further symbols and abbreviations used herein have the following meanings:

Aa	=	amino acid
Aa^p	=	phosphonic acid analogue of Aa
Ac	=	acetyl
adal	=	adamantylalanine

	Adgly	=	1-adamantylglycine
	Apa	=	amidinophenylalanine
	ArgCN	=	Arg, where COOH is replaced by CN
	Baa	=	NH-CH-($CH_2CH_2CH_2Br$)B-
5	Boc	=	t-butyloxycarbonyl
	BoroArg	=	NH-CH-[$CH_2CH_2CH_2NHC(NH)NH_2$]B
	BoroHArg	=	NH-CH-($CH_2CH_2CH_2CH_2NHC(NH)NH_2$)B
	BoroHpg	=	borohydroxypropylglycine
	$COCH_2$boroHpg	=	-$COCH_2$-CH($CH_2CH_2CH_2OH$)B
10	BoroLys	=	NH-CH-($CH_2CH_2CH_2CH_2NH_2$)B
	BoroOrn	=	NH-CH-($CH_2CH_2CH_2NH_2$)B
	BoroPro	=	analogue of proline in which the -COOH group is replaced by BO_2Pin
	BPoc	=	biphenyl methyl oxycarbonyl
15	Bu	=	butyl
	Bz	=	benzoyl
	Bzl	=	benzyl
	Cbz	=	benzyloxycarbonyl
	Ceg	=	chloroethylglycine
20	Cha	=	cyclohexylalanine
	Chg	=	cyclohexylglycine
	Dba	=	α-phenylethylphenylalanine
	DBU	=	diaza-bicycloundecane
	DCC	=	dicyclohexylcarbodiimide
25	DCM	=	dichloromethane
	DCU	=	dicyclohexylurea
	DIEA	=	diisopropylethylamine
	DMAP	=	4-dimethylaminopyridine
	Dpa	=	β,β-diphenylalanine
30	Dtt	=	dithiothreitol
	ESMS	=	electrospray mass spectrometry

	Etg	=	α-ethylglycine
	EtOAc	=	ethyl acetate
	EtOH	=	ethylalcohol
	Fgl	=	α-fluorenylglycine
5	Gpa	=	guanidinophenylalanine
	HOBT	=	Hydroxybenzotriazole
	Irg	=	isothiouronium analogue of Arg
	-k-	=	amide bond replaced by $CO\text{-}CH_2$
	LDA	=	lithium diisopropyl amide
10	Mbg	=	2-(2-methylbutyl) glycine
	Mpg	=	3-methoxypropylglycine
	MCA	=	4-methyl-coumaryl-7-amide
	MeOH	=	methylalcohol
	MeOSuc	=	methoxysuccinyl
15	Mtr	=	4-methoxy-2,3,6-trimethylbenzenesulphonyl
	Nal	=	naphthylalanine
	$NaSO_2$	=	napthylsulfonyl
	NMR	=	nuclear magnetic resonance
	Np	=	p-nitrophenyl
20	ONSu	=	N-hydroxysuccinimide
	OPin	=	pinanediol
	OPinac	=	pinacol
	PfpOH	=	pentafluorophenol
	Phg	=	phenylglycine
25	Pgl	=	pentylglycine
	pip	=	piperidide
	Pmc	=	2,2,5,7,8-pentamethylchroman-6-sulphate
	Pms	=	phenyllactic acid
	pNA	=	p-nitroanilide
30	Pyro	=	pyro-glutamic acid
	p-OH-Me-Phe	=	p-hydroxymethylphenylalanine

p-TBDPS-	=	p-tertbutyldiphenylsilyl
rmm	=	relative molecular mass
TEA	=	triethylamine
TFA	=	trifluoroacetic acid
THF	=	tetrahydrofuran
Thi	=	thiazolidinecarboxylic acid
Tiq	=	tetrahydroisoquinoline-3-carboxylic acid
TLC	=	thin layer chromatography
TMSal	=	trimethylsilylalanine
WSC	=	water soluble carbodiimide

The term "aryl" as used herein includes aryl groups containing heteroatoms, i.e. heteroaryl groups.

The term "alkyl" includes cycloalkyl and alkyl containing cycloalkyl, where cycloalkyl is in particular cyclohexyl or cyclopentyl.

As used herein, "amino protecting group" means any amino protecting group employable in peptide synthesis. Examples include: alkyl (especially methyl or other C_1-C_6 alkyl), acetyl, benzoyl, BPoc, formyl, morpholinocarbonyl, trifluoroacetyl, methoxysuccinyl, aromatic urethane protecting groups such as benzyloxycarbonyl, aliphatic urethane protecting groups such as tert-butyloxycarbonyl or adamantyloxycarbonyl. Amino protecting groups are described in Gross and Meinhoffer, eds., The Peptides, Vol. 3, 3-88, and exemplified in D. W. Greene, "Protecting Groups in Organic Synthesis".

Preferred amino protecting groups include: $R^{10}(CH_2)_eOCO$- or $R^{10}(CH_2)_eSO_2$-, where R^{10} is a C_5-C_{12}, preferably C_6-C_{10}, aryl, arylalkyl or alkylaryl group optionally substituted by halogen or -OH, especially phenyl, naphthyl or C_1-C_4 alkylphenyl, and e is 0 to 3.

In compounds of the invention having side chain amino groups, e.g. where aa^1, aa^2, aa^3 or aa^4 is Lys or Arg, additional N-protecting groups are desirable in the compound structure during synthesis. These protecting groups are optionally removed or exchanged in the final structure. For example Mtr (4-methoxy-2,3,6-trimethyl-benzenesulphonyl) or Pmc (2,2,5,7,8-pentamethyl-chroman-6-sulphate) may be used to protect Arg and Dtt (dithiothreitol) to protect Lys.

Similarly amino acid residues having acidic or hydroxy side chains may be suitably protected in the form of t-butyl, benzyl or other suitable esters or ethers, as is known in the art (e.g. Sheppard - "Solid Phase Peptide Synthesis, E. Atherton, R.C. Sheppard, IRL Press, Oxford, 1989).

Besides the true acid forms of the peptides of the above formula (I), within the scope of the present invention are also physiologically acceptable salts thereof. Preferred salts include acid addition salts, e.g. salts of benzene sulphonic acid (BSA), hydrochloric acid (HCl), hydrobromic acid (HBr), acetic acid, trifluoroacetic acid (TFA), succinic acid, citric acid and other addition salt-forming acids known in the art.

Within the scope of the present invention are inhibitors modified by, in particular, isosteric replacement of one or more peptide bonds by $-CO-CH_2-$, $-CH(OH)-CH_2$ or $-CH_2-NH-$ linkages, or by N_4. The peptides may be in the free form or in a form protected at one or more remaining functional groups, e.g. amino, imino or amide (including peptide), nitro, carboxyl, hydroxyl, guanidino or nitrile. Examples of, and synthetic routes to, such further modifications of peptides are disclosed in for example EP-A-0118280, corresponding US patents 4638047 and 4772686 , the disclosures of both of which references are incorporated herein by references, as well as in WO 92/07869 and PCT/GB96/00352.

In one aspect, therefore, the present invention provides thrombin inhibitors comprising:

a) a catalytic site-directed moiety (CSDM) that binds to and inhibits the active site of thrombin;

b) an anion binding exosite associating moiety (ABEAM); and

c) a connector moiety bonded to the CSDM as an N-terminal extension or as or through a side chain thereof and to the ABEAM, the connector moiety being capable of permitting the CSDM and the ABEAM to bind simultaneously to a molecule of thrombin.

The invention also provides a preferred class of compounds of the formula:

$$\sigma\text{-}\alpha_1\text{-}\alpha_2\text{-}\alpha_3\text{-}\alpha_4\text{-}\alpha_5\text{-}\alpha_6\text{-}\alpha_7\text{-}\alpha_8\text{-}\alpha_9\text{-}(\alpha_{10})_{w1}\text{-}(\alpha_{11})_{w2}\text{-}(\alpha_{12})_{w3}\text{-}\Omega$$
$$\lambda\text{-CO-}(aa^4)_m\text{-}(aa^3)_n\text{-}(aa^2)\text{-}(aa^1)\text{-Z,}$$

wherein: the moiety α_1 ....Ω represents an ABEAM and is preferably as defined herein under the heading "The Anion Binding Exosite Associating Moiety (ABEAM)"; σ is a peptide spacer and λ is a non-peptide linker, each preferably being as defined herein under the heading "The Connector Moiety"; and $(aa^4)_m$ ... Z represents the CSDM and is preferably as defined herein under the heading "The Catalytic Site-Directed Moiety (CSDM)".

In a less preferred class of compounds the positions of the σ and λ moieties in the above formula are exchanged.

Brief Description of the Drawing

The Figure is a schematic representation of the binding of an inhibitor of the invention to thrombin.

Considering now the inventive compounds in more detail:

The Catalytic Site-Directed Moiety (CSDM)

The catalytic site directed moiety (CSDM) binds to and inactivates the catalytic site of thrombin. The structure of the CSDM is not critical to the invention. It may comprise the amino acid sequence of any known inhibitor of the thrombin catalytic site, for example. Suitable CSDMs may comprise a peptide inhibitor of formulae I or II, in which one or more natural amide linkages may be replaced by an alternative linkage, for example the P1-P2 link as described in International patent application No PCT/GB96/00352 and shown in Formulae III and IV.

Preferred CSDMs are peptides in which the C-terminus carboxy group is replaced by a heteroatom acid group or a derivative thereof, especially a boron-containing group. Suitable CSDMs of this class are described in WO 92/07869 (and corresponding USSN 08/317,837), US 5288707, US 5196404 and PCT/GB96/00352 and corresponding cases. The most preferred CSDMs contain the amino acid sequence Phe-Pro-Arg or sequences in which one or more of Phe, Pro and Arg is replaced by, for example, a known functional alternative or analogue. Notably, Phe may be replaced by another hydrophobic amino acid, such as Dpa, Dba or Nal, whilst Arg may be replaced by a residue with an alkoxyalkyl side chain or a side chain having a terminal amino, amidino, imidazole, guanidino, N_3 or isothioureido group. Mpg is a preferred Arg replacement. The structures of some suitable CSDMs will be elucidated further in the following paragraphs.

As already indicated, therefore, suitable CSDMs comprise, amongst other structures, peptides falling within formulae I, II, III and IV:

$$X\text{-}(aa^4)_m\text{-}(aa^3)_n\text{-}(aa^2)\text{-}(aa^1)\text{-}Z, \qquad I$$

$$W\text{-}A\text{-}Z \qquad II$$

$$X\text{-}(aa^4)_m\text{-}(aa^3)_n\text{-}(aa^2)\text{-}\psi\text{-}(aa^1)\text{-}Z \qquad III,$$

$$W\text{-}\psi\text{-}A\text{-}Z \qquad IV$$

which peptides are in the compounds of the invention bonded to a connector moiety other than through Z, which is C-terminal carboxy group (residue) or a replacement therefor. In formulae I and III, m is normally from 0 to 7 and n is 0 or, most usually, 1.

Considering now these formulae in more detail:

The C-Terminal Group (Z)

The moiety on the C-terminal side of the P1 residue (groups Z of formulae I and II) is not critical to the invention and may be any such moiety known in the art, for example. It is a moiety which interacts with the active site triad residues (Asp-His-Ser) of thrombin. Typically, the P1 residue is linked on its C-terminal side to a functional group which may be a carboxyl group (-COOH) or a derivative thereof, such as an ester, an amide or a ketone, for example, or even a nitrile group. Preferably the natural carboxy group is replaced by a heteroatom acid group, of which the preferred examples are boron or phosphorus acid groups, notably boronic acid residues [$-B(OH)_2$], phosphonic acid residues [$-P(O)(OH)_2$], phosphorous acid residues [$-P(OH_2)$] or phosphinic acid residues [-P(O)(OH)(H)]. The boron groups are most preferred.

A less preferred heteroatom acid group is sulphonyl [$-S(O)_2OH$].

In place of a heteroatom acid group there may be used a derivative thereof. The invention is not primarily concerned with selection of derivatives of the carboxy or heteroatom acid groups: in principle, any derivative group may be used which does not prevent the inhibiting function of the compound. Substituent groups include inert organic groups, generally containing a total number of carbon atoms and heteroatoms

not exceeding 20. Representative inert groups are hydrocarbyl, optionally containing an ether or ester linkage and/or substituted by halogen or -OH.

In one class of embodiments, the acid derivatives have the hydrogen of an -OH group replaced by a substituent group, which may be linked to the oxygen by a functional group, for example a carbonyl or amino group. Preferred substituents are diol residues, as further described below.

In another class of embodiments an -OH group is replaced by an amino group, which may be mono- or di- substituted. An alternative replacement functional group is thiol, especially substituted thiol.

In other classes of compounds, an -OH group is replaced by an inert organic group (e.g. a hydrocarbyl group as described above) or by a halogen atom, especially fluorine.

One class of CSDMs has a C-terminal group (Z of formulae I - IV) of the formula V:

$$-Het(O)_s(Y)_{t-2s} \qquad V$$

wherein

Het is a heteroatom;

s is 0, 1 or 2;

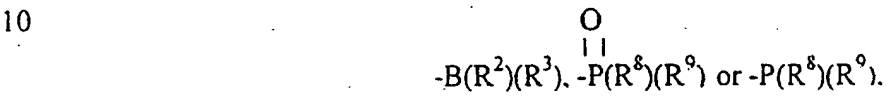
t is the valency of Het, t-2s being an integer of at least 1; and

each Y is independently hydrogen, halogen, hydroxy, substituted hydroxy, substituted thiol, amino or substituted amino, wherein two hydroxy groups, two thiol groups or an amino group are/is optionally substituted by a single divalent substituent.

Het is preferably boron or phosphorus, and is most preferably boron.

Preferably, each Y is independently F or other halogen, $O\Sigma^1$ or $N\Sigma^1\Sigma^2$, wherein Σ^1 and Σ^2 are independently selected from H, hydrocarbyl and hydrocarbylcarbonyl, the hydrocarbyl groups optionally being substituted by one or more moieties selected from halogen, -OH or alkoxy and/or containing an ether or ester linkage (-O- or -COO-), which groups contain up to 20 carbon atoms, or wherein two Y groups taken together form the residue of a diol or a dithiol.

Particularly preferred C-terminal groups are of the formula



$-B(R^2)(R^3)$, $-P(R^8)(R^9)$ or $-P(R^8)(R^9)$.

wherein: R^2 and R^3 are each independently selected from halogen, -OH, $-OR^4$ and $-NR^4R^5$, where R^4 and R^5 are each independently a group of the formula $R^6(CO)_u$-, wherein u is 0 or 1. R^6 is H or an optionally halogenated alkyl, aryl or arylalkyl group containing up to (10 - u) carbon atoms and optionally substituted by one or more groups selected from -OH, $R^7(CO)_vO$- and $R^7(CO)_v$-, wherein v is 0 or 1. R^7 is C_1-C_{6-v} alkyl, or is an aryl, alkylaryl, arylalkyl or alkylarylalkyl group containing up to (10-v) carbon atoms,

or R^2 and R^3 taken together represent a residue of a diol or a dithiol:

R^7 and R^8 and are each independently selected from the group consisting of R^2, R^3, R^4 and R^5; and

R^9 is a group selected from the following: -H, $-OR^4$, $-OR^5$.

One or both of R^2 and R^3 are preferably $-OR^4$ in which R^4 is preferably a said optionally halogenated alkyl, aryl or arylalkyl group optionally substituted as aforesaid.

Where the compounds have a C-terminal acid group substituted by the residue of a diol or dithiol, the diol or dithiol preferably comprises two or more -OH or, as the case may be, -SH groups connected by at least two connecting atoms. The connecting atoms are preferably in an organic moiety containing up to 20 and, more preferably, up to 10 carbon atoms.

The organic moiety may be a hydrocarbyl group optionally containing between the members of one or two pairs of adjacent carbon atoms an N, S or O atom. The organic moiety may be inertly substituted. Normally the substituted compounds are mono- or di- substituted, exemplary substituents being halogen especially -F, and -OH.

Preferred diol residues are of pinanediol, pinacol, perfluoropinacol, ethylene glycol, diethylene glycol, catechol, 1,2-cyclohexanediol, 1,2-cyclohexaneethanediol, 1,3-propanediol, 2,3-butanediol, 1,2-butanediol, 1,4-butanediol, 2,3-dimethylbutane-2-3-diol, glycerol, or diethanolamine or another amino dihydroxy alcohol. Of these, pinanediol and especially pinacol are most preferred. The most preferred compounds comprise a boronic acid residue substituted with a diol residue.

The Optional Replacement Non-Amide Bond (ψ)

The compounds of the invention optionally may contain a CSDM in which a natural peptide linkage (-NHCO-) is replaced by an alternative linker group. The replaced peptide link may be the P1-P2 link or another link and is represented by ψ in formulae III and IV. For convenience, the symbol ψ will hereafter be used.

ψ is a group which may be included in a compound of the invention without the inhibiting activity of the compound being lost . Preferred ψ groups enhance the inhibitory activity of the compound. If ψ is long, there is a tendency for binding of the peptide inhibitor to the target enzyme to be weakened. Typically, therefore, ψ has a chain length of no more than 5 atoms, i.e. no more than 5 atoms separate the carbon

atoms of the residues linked by ψ. More preferred ψ groups have a chain length of 2 or 3 atoms, a chain length of two atoms being most preferred.

ψ is preferably not isoelectronic with -NHCO- of it forms the P1-P2 linkage. One less preferred class of embodiments does not have ψ groups of the so-called isosteric (to -CONH-) type, such as $-COCH_2-$, $-CH(OH)-CH_2-$, $-CH_2-NX-$ or -NHCO-, for example. However, $-COCH_2-$ and $-CH(OH)-CH_2-$ are very acceptable in some compounds. Representative ψ groups include $-CO_2-$, $-CH_2O-$, -NHCO-, $-CHYCH_2-$, -CH=CH-$CO(CH_2)_pCO-$ where p is 1, 2 or 3, $-COCHY-$, $-CO_2-CH_2NH-$, $-CHY-NX-$, $-N(X)CH_2-$ N(X)CO-, -CH=C(CN)CO-, -CH(OH)-NH-, -CH(CN)-NH-, $-CH(OH)-CH_2$ or -NH-CHOH-, where X is H, an amino protecting group (e.g. CH_3) and Y is H or halogen (especially F). Exemplary Y-containing groups are $-CH_2CH_2-$, -COCHF- and $-CH_2NX-$. The most preferred ψ groups are $-CO_2-$ and $-CH_2O-$.

It is known in the art to replace other amide linkages than the P1-P2 link with unnatural replacements, especially so-called isosteric/isoelectronic linkers. The invention encompasses peptides in which one or more amide linkages other than the P1-P2 linkage are also replaced by an unnatural linker ψ, e.g. a preferred ψ group of this invention or, more preferably, a so-called isosteric group, e.g. $-COCH_2-$, $-CH(OH)-CH_2-$ or $-CH_2-NH-$. Such replacement of peptide bonds other than the P1-P2 bond is described, for example, in EP 0118280.

The N-terminal Group (X)

The N-terminal group (X of the Formulae) is in many compounds of the invention replaced by the connector moiety and its attached ABEAM. Where present, X may be hydrogen (to form an $-NH_2$ group) or an amino protecting group. The amino protecting group of the pharmaceutical compounds may be any pharmaceutically acceptable group, for example as described hereinbefore. Alkyl groups, e.g. C_1-C_6 alkyl such as methyl, for example, are suitable. A preferred class of protecting groups are those of the

formula $R^{10}(CH_2)_eOCO$- and $R^{10}(CH_2)_eSO_2$-. wherein e is 0, 1, 2 or 3 and R^{10} is a C_5-C_{12} aryl, C_5-C_{12} arylalkyl or C_5-C_{12} alkylaryl group optionally substituted by halogen (e.g. -F or -Cl) or -OH; such protecting groups are especially preferred when m and n of Formula I are both 0, and are described in more detail hereafter in relation to compounds in which m and n are both 0 under the heading "The Amino Acid Sequence". Particularly preferred R^{10} groups when m and n are 0, or when m is 0 and n is 1, are phenyl, naphthyl, C_1-C_4 alkylphenyl or phenyl C_1-C_4 alkyl. In preferred embodiments, e is 0.

Selection of N-terminal groups can enhance bioavailability of active compounds, although not necessarily effecting potency against the isolated target enzyme. Typical groups of the active compounds include morpholin-N-alkyl or N-carbonyl derivatives, succinimidyl, alkyl or aryl-alkyl-sulphonyl, N-methylpiperazine or groups as known in the art, such as Rosenberg, et al. *J.Med.Chem.*, 1993, 36, 449-459 or Hashimoto, N. et al. *Pharm.Res.*, 1994, 11, 1443-1451, or Bernstein, P.R., et al. *J.Med.Chem.*, 1994, 37, 3313-3326. These groups can be introduced to the peptides by hydrogenation to remove urethane protecting groups used for synthesis to give the free amino terminus (see Example 2) and reacylation or acetylation with a derivative of the appropriate X group.

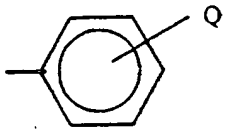
Introduction of N-methyl groups can improve in-vivo activity as is known in the art. Hashimoto, N. et al *Pharm.Res.*, 1994, 11, 1443-1451.

The Amino Acid Sequence

Peptide inhibitors of the thrombin catalytic site comprise a sequence of amino acid residues and are commonly tripeptides. The specific sequence is not critical to the invention. The amino acids may be natural or unnatural, e.g. the D-isomer or racemate of a natural amino acid; they may be modified amino acids in which the α-H is replaced by a substituent, for example hydrophobic or hydrophilic groups containing up to about

20 or even more, e.g. 22, carbon atoms. More preferred substituents contain up to 15, or preferably up to 10, carbon atoms.

Preferred classes of replacements for the α-hydrogen of the amino acid residues are:

(i) C_2H_{2q}-Q or C_qH_{2q} 

where Q = amino, amidino, imidazole, guanidino, N_3, or isothioureido, and q is an integer of from 1 to 5;

(ii) a side chain of a natural amino acid other than glycine; or

(iii) a moiety (other than hydrogen) of the formula V or VI:

$$-(CO)_a-(CH_2)_b-D_c-(CH_2)_d-E \qquad V$$

$$-(CO)_a-(CH_2)_b-D_c-(CH)_e(E^1)(E^2) \qquad VI$$

wherein:

a is 0 or 1;

e is 1;

b and d are independently 0 or an integer such that (b+d) is from 0 to 4 and (b-e) is from 1 to 4;

c is 0 or 1;

D is O or S;

E is H, C_1-C_6 alkyl, or a saturated or unsaturated cyclic group which normally contains up to 14 members and preferably is a 5-6 membered ring or an 8-14 membered fused ring system, which alkyl or cyclic group is optionally substituted by up to 3 groups (e.g. 1 group) independently selected from $-R^{13}$, $-R^1OR^{13}$, $-R^1COR^{13}$, $-R^1CO_2R^{13}$, $-R^1O_2CR^{13}$, nitro and cyano, wherein R^1 is $-(CH_2)_f-$ and R^{13} is $-(CH_2)_gH$ or a moiety which has a total number of carbon and

heteroatoms from 5 to 10 and which contains a ring system (e.g. an aryl group) and optionally an alkyl and/or alkylene group, wherein f and g are each independently from 0 to 10. g preferably being at least 1 except that -OH is a preferred substituent, provided that (f+g) does not exceed 10. preferably does not exceed 6 and more preferably is 1, 2, 3 or 4, and provided that there is only a single substituent if the substituent is a said moiety containing a ring system, or E is C_1-C_6 trialkylsilyl: and E^1 and E^2 are each independently a 5 or 6 membered ring;

in which moiety of Formula V or VI any one or more hydrogen atoms bonded to a carbon atom is optionally replaced by halogen, especially F.

Certain classes of compounds falling within definition (iii) above are preferred. Preferably a is 0. If a is 1, c is preferably 0. Preferably, (a+b+c+d) and (a+b+c+e) are no more than 4 and are more preferably 1, 2 or 3. (a+b+c+d) may be 0.

Exemplary groups for E, E^1 and E^2 include aromatic rings such as phenyl, naphthyl, pyridyl, quinolinyl and furanyl, for example; non-aromatic unsaturated rings, for example cyclohexenyl; saturated rings such as cyclohexyl, for example: and fused ring systems containing both aromatic and non-aromatic rings, for example fluorenyl. A preferred class of E, E_1 and E^2 groups are aromatic rings, especially 6- membered aromatic rings. E^1 and E^2 are preferably phenyl. The phenyl or other aryl groups may be substituted by nitro or cyano, preferably at the 4-position.

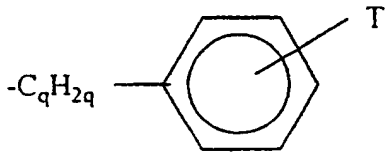
In one class of embodiments, E contains a substituent which is C_1-C_6 alkyl, (C_1-C_5 alkyl)carbonyl, carboxy C_1-C_5 alkyl, aryl, especially 5-membered or preferably 6-membered aryl (e.g. phenyl or pyridyl), or arylalkyl (e.g. arylmethyl or arylethyl where aryl is preferably 6-membered).

In another class of embodiments, E contains a substituent which is OR^{13}, wherein R^{13} preferably is a 6-membered ring, which may be aromatic (e.g. phenyl) or non-aromatic

(e.g. morpholine or piperazine) or is alkyl (e.g. methyl or ethyl) substituted by such a 6-membered ring.

A particularly preferred class of moieties of formula V or VI are those in which E is a 6-membered aromatic ring substituted, preferably at the 2-position or 4-position, by $-R^{13}$ or $-OR^{13}$.

A further preferred class of substituents of formula V or VI are of the formula $C_qH_{2q}T$ or



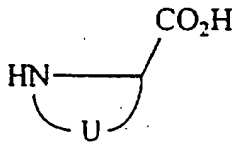
wherein q is as defined above and T is hydrogen, halogen (e.g. F), $-SiMe_3$, $-R^{13}$, $-COR^{13}$, CO_2R^{13}, $-O_2CR^{13}$ or a moiety which has a total number of heteroatoms from 5 to 10 and which contains a ring system, especially an aryl group, and optionally an alkyl residue or an alkylene residue, or both. Said moiety is preferably 5-membered or more preferably 6-membered aryl (e.g. phenyl or pyridyl) or arylalkyl (e.g. arylmethyl or arylethyl) where aryl has 5 or preferably 6 members. In preferred embodiments T is at the 2-position of the phenyl group and is $-R^{13}$, $-COR^{13}$, $-CO_2R^{13}$ or $-O_2CR^{13}$, and R^{13} is C_1-C_{10} alkyl and more preferably C_1-C_6 alkyl.

A class of residues which includes certain natural amino acid residues as well as many unnatural amino acid residues is of the formula

$$-HNC(W^1)(W^2)CO-,$$

wherein W^1 and W^2 may be the same or different and are selected from hydrogen and hydrogen replacement groups (i), (ii) and (iii) described above in relation to amino acid residues in which the α-hydrogen is replaced by a substituent; preferably, one of W^1 and W^2 is hydrogen. In other residues of this formula, W^1 and W^2 together with the carbon atom to which they are bonded form a ring system, especially a hydrophobic ring system such as cycloalkyl (e.g. C_3-C_7 cycloalkyl) or W^1 and W^2 together form an alkenyl or

aralkenyl group, e.g. PhCH=. Alternatively, -HNC(W^1)(W^2)CO- is the residue of an amino acid in which W^1 is H and W^2 is a group which together with the α-amino group forms a cyclic group, i.e. the amino acid is of the formula IX:



IX

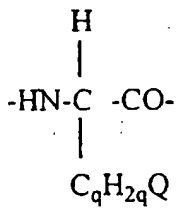
wherein U is a moiety forming a cyclic structure, which may be substituted or unsubstituted. The cyclic structure is preferably a 4-6 membered ring or an 8-10 membered fused ring system optionally substituted by up to 3 groups independently selected from $-R^{13}$, $-R^1OR^{13}$, $-R^1COR^{13}$, $-R^1CO_2R^{13}$ and $-R^1O_2CR^{13}$, wherein R^1 and R^{13} are as hereinbefore defined. Exemplary substituents are C_1-C_3 alkyl. Any one or more hydrogen atoms bonded to a carbon atom may optionally be replaced by halogen, especially F.

The cyclic structure may contain additional heteroatoms, for example sulphur, such as in a 5- or 6- member ring, for example. A ring carbon atom may be a member of a carbonyl group, for example as part of an amide linkage in the cyclic structure, as in pyroglutamic acid, for example. In one preferred class of compounds the cyclic structure preferably contains no heteroatom in addition to the α-amino nitrogen. In fused ring structures, the ring fused to that containing the α-amino nitrogen is preferably aromatic and most preferably phenyl, as in D-Tiq.

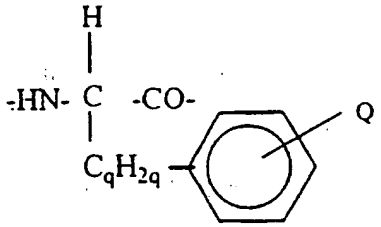
The number of aa^4 residues is not critical to the invention but in preferred embodiments m is from 0 to 7 and more usually 0 to 5, e.g. 0, 1 or 2 especially 0. Normally there is an aa^3 residue (i.e. n=1) but if X is a suitable group m and n may both be zero.

The P1 Residue

Residues analogous to Lys or Arg and amongst the residues favoured by thrombin at P1 (i.e. as aa^1) are those with group (i) side chains and an α-hydrogen, that is, residues of the formula



and

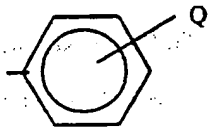


in which Q includes amino, amidino, imidazole, guanidino, N_3 or isothioureido. Specific analogy residues to Lys and Arg include Gpa, amidinoPgl or amidinopiperidylglycine. Also very acceptable P1 residues are those with hydrophobic side chains, including Phe and its analogues.

Suitable hydrophobic side chains for the P1 residue include group (iii) side chains of Formula V, especially those in which a is 0, D is O or is absent and/or E is H, C_1-C_6 alkyl, C_1-C_6 trialkylsilyl or C_6-C_{10} aryl optionally substituted by up to three groups selected from C_1-C_4 alkyl, halogen and C_1-C_4 alkoxy, of which H is less preferred. Preferably there is one said substituent; preferably the Formula V groups contain a total number of carbon atoms and heteroatoms not exceeding 14, more preferably not exceeding 10 and most preferably not exceeding 8.

It will therefore be seen that, as suitable P1 residues there, may be mentioned groups of the formula -CH-
J

wherein

J is $-C_qH_{2q}-Q$ or C_qH_{2q} 

where Q and q are as defined above or is a group of the formula

$$-(CH_2)_b-D_c-(CH_2)_d-E$$

wherein:

b, d, c and e are as defined above;

D is 0; and

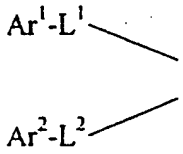
E is H, C_1-C_6 alkyl, C_1-C_6 trialkylsilyl or C_6-C_{10} aryl optionally substituted by one or, less preferably, two or three groups selected from C_1-C_4 alkyl, halogen and C_1-C_4 alkoxy. C_1-C_6 haloalkyl is a preferred E group.

Particularly preferred hydrophobic P1 side chains are C_1-C_8, preferably C_1-C_6, alkyl (e.g. ethyl, isopropyl, pentyl), alkoxyalkyl containing from 2 to 6 carbon atoms (e.g. methoxypropyl) and moieties containing a 5-10 membered aryl or heteroaryl group and optionally a total number of alkyl and/or alkylene carbon atoms not exceeding 4, especially phenyl C_1-C_4 alkyl (e.g. phenylmethyl). Any of the aforesaid alkyl or alkylene groups may be substituted by one, or more than one, halo atom, e.g. fluoro or bromo; thus bromopropyl, especially 3-bromopropyl, or other bromoalkyl (usually substituted by Br at the terminal carbon) is a preferred P1 side chain. Methoxyalkyl is a particularly preferred side chain. In some embodiments the P1 side chain is C_1-C_6 hydroxyalkyl. 3-methoxypropyl, 3-halopropyl and 3-hydroxypropyl and alkyl homologues thereof are particularly preferred.

The P2 and P3 Residues

W of formula II normally comprises a sequence of up to 9 amino acids, and more usually of up to 7 amino acids, wherein at least one amino acid has a hydrophobic side chain, e.g. Phe or a Phe analogue. For thrombin inhibitors the P3 residue (aa^3) is desirably hydrophobic; the P2 residue (aa^2) is also preferably hydrophobic and more preferably is Pro or a ring homologue thereof. Any P4 residue of a thrombin inhibitor is preferably also hydrophobic.

Residues analogous to Phe include those with group (iii) side chains, those of formula IX and those in W^1 and W^2 from a hydrophobic ring system or an alkenyl or aralkenyl group. A class of Phe analogues with group (iii) side chains or of formula IX, which class is in particular preferred for the P2 and especially P3 residues, comprises compounds of the Formula VII:



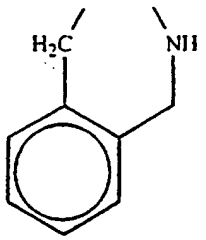
VII

wherein

Ar^1 and Ar^2 are each independently selected from the group consisting of H; phenyl; phenyl substituted by halogen (e.g. p-halophenyl, especially p-iodophenyl), a C_1-C_6 group which is alkyl or alkyl substituted or interrupted by a carbonyl or carbonyloxy group (e.g. alkylcarbonyl or alkoxycarbonyl) or substituted by $-R^{14}$ or $-OR^{14}$ wherein R^{14} is a 5- or 6-membered aromatic or non-aromatic ring or is C_1-C_4 alkyl substituted by such a 6-membered ring; bipyridyl; furanyl; chromanyl; quinolinyl; thienyl; pyridyl; α- or β-naphthyl; thionaphthyl; indolyl; p-iodophenylalanyl; diphenyl-methyl; or fluorenyl; or are wholly or partially saturated groups corresponding to any of these (e.g. cyclohexyl, piperidyl or tetrahydroisoquinolyl); Me_3Si, or 2,2,2-trichloroethyl. Any of the foregoing groups is optionally substituted by up to three groups selected from C_1-C_3 alkyl, C_1-C_3 alkoxy or $R^{13a}CO$- wherein R^{13a} is H, CH_3 or C_2H_5, or by a group of the formula R^1-O-, wherein R^1 is as hereinbefore defined.

L_1 and L_2 are each independently selected from the group consisting of CH_2, CH_2-CH_2, O-CH_2, S-CH_2, and a bond.

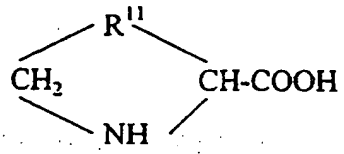
V is H, or -NHV and one of Ar^1-L^1 and Ar^2-L^2 together form a group of the formula



It is preferred that, if L^1 or L^2 is a single bond, its attached Ar group be diphenylmethyl, fluorenyl or cyclohexyl.

Preferably Ar^2-L^2 is H.

The P2 (aa^2) residue is normally proline or an analogous residue. Residues analogous to proline are preferably those ring homologues included in the formula VIII



VIII,

or its C_1-C_3 alkyl substituted derivatives. where R^{11} = -CH_2-, -CH_2-CH_2-, -S-CH_2-, -S-$C(CH_3)_2$- or -CH_2-CH_2-CH_2-. Up to 3 C_1-C_3 alkyl groups, e.g. methyl, may substitute one or more carbon atoms. Normally any substituent is on a -CH_2- group. Normally a -CH_2- group is substituted by no more than 1 alkyl group.

[Formula VIII = proline when R^{11} = -CH_2-CH_2-].

Particularly preferred proline analogues are 2- and 3-thioproline and pipecolic acid.

Particularly preferred Phe analogues for the P3 residues are D-Phe substituted at the phenyl 2-position (i) by a C_1-C_6 group which is alkyl or alkyl substituted or interrupted by a carbonyl or carbonyloxy group (e.g. is alkylcarbonyl or alkyloxycarbonyl) or (ii) by a 5 or 6 membered aryl group; D-Dpa; Dba; Pms; α- or β-Nal; TMSal; Chg; Phg; D-

Tiq or a para ether of D-Tyr. An exemplary substituted phenylalanine residue is D-phenylalanine-2-carboxylic acid methyl ester. Exemplary tyrosine-para-ethers are D-tyrosine-O-phenyl. D-tyrosine-O-ethyl-2-(N-morpholine) and D-tyrosine-O-ethyl-2-N(piperazine). The most preferred Phe analogues are Dpa, Nal and Dba. Other preferred Phe analogues for. in particular, the P3 residue have side chain c), d), e) f), g), or h) of US 5288707 and EP 0471651.

As a modification of tripeptides in which the P3 residue is in particular Phe or another hydrophobic residue (e.g. Mpg), there may be used a dipeptide (m and n = 0 in Formula I) in which X is of the formula $R^{10}(CH_2)_eCOO-$ or $R^{10}(CH_2)_eSO_2-$ wherein R^{10} is a C_5-C_{12} aryl, C_5-C_{12} arylalkyl or C_5-C_{12} alkylaryl group optionally substituted by halogen or -OH and e is 0 to 3. Particularly preferred R^{10} groups are C_9-C_{10} fused ring systems containing a phenyl ring, especially naphthyl. Where R^{10} is a fused ring system, e is preferably 0; if R^{10} is a single ring, e may suitably be 1. In those compounds in which R^{10} is a fused ring system, the residue of the acid function -COO- or $-SO_2-$ is preferably $-SO_2-$. Particularly preferred amino protecting group analogues for Phe are benzyloxycarbonyl (Cbz) and naphthylsulfonyl.

Those residues which are an analogue of Phe, Arg or Lys preferably have an α-hydrogen, but the hydrogen may be replaced by another group, e.g. a W moiety.

As already indicated, preferred classes of P1 (aa^1) residues of the inventive compounds are (i) Arg, Lys and their analogues as described above, and (ii) hydrophobic residues.

Preferred P2 (aa^2) residues are Pro and its analogues as described above. Preferred P3 (aa^3) residues are Phe and its analogues as described above.

Tripeptide inhibitors are preferred, especially tripeptide boronates, and a particularly preferred sequence is PheProBoroMpg, in which one or more natural peptide bonds may be replaced by another link. The P1 Mpg residue may be replaced by Pgl or Bpg.

Residues may be in either D- or L-configuration. D-configuration is preferred for the P3 residue of thrombin inhibitors.

The Anion Binding Exosite Associating Moiety (ABEAM)

The anion binding exosite associating moiety (ABEAM) is a moiety which binds to the anion binding exosite (ABE) of thrombin. It preferably comprises an amino acid sequence of the ABE binding domain of hirudin or an ABE binding modification thereof.

In a first class of compounds the ABEAM comprises a sequence of up to 15 amino acids of which at least four are anionic amino acids and more normally at least five or six are anionic. Preferably, the ABEAM of this class comprises a sequence of up to 13 amino acids, e.g. of 10, 11, 12 or 13 amino acids.

In a second class of compounds the ABEAM comprises a sequence of up to 10 amino acids e.g. of 8, 9 or 10 amino acids of which at least 3 amino acids are anionic and preferably 4 or 5 are anionic. An exemplary sequence is EDFEPIPL.

In both the aforegoing classes of compounds, the anionic amino acids preferably include residues 3 and 4 from the C-terminus. or residues 4 and 5 from the C-terminus. The invention includes compounds in which all of residues 3, 4 and 5 from the C-terminus are anionic.

Particularly preferred ABEAMs comprise a moiety of the following formula:

$$-\alpha_1-\alpha_2-\alpha_3-\alpha_4-\alpha_5-\alpha_6-\alpha_7-\alpha_8-\alpha_9-(\alpha_{10})_{w1}-(\alpha_{11})_{w2}-(\alpha_{12})_{w3}-\Omega$$

wherein each of α_1 to α_{12} is an amino acid residue, w1, w2 and w3 are each independently 1 or 0 (but w3 is preferably 0) and Ω is a carboxy terminal residue

(attached to the terminal carbonyl group) preferably selected from hydroxy, C_1-C_6 alkoxy, amino, mono- or di (C_1-C_4) alkyl substituted amino or benzylamino, in which moiety α_4, α_8 and α_9 and two or three other amino acid residues are anionic. Preferably said other anionic amino acid residues include α_1 or α_2. Preferably said moiety includes at least two pairs of adjacent anionic amino acid residues. Thus, exemplary ABEAM moieties have anionic amino acids as α_4, α_5, α_8, α_9 and preferably, α_2. Other exemplary ABEAM moieties have anionic amino acids as α_8-α_{11}, α_1 and α_4.

The anionic amino acids are desirably Gly or Asp. Ω is preferably hydroxy.

The invention includes compounds containing an ABEAM comprising the α_4-α_{11} sequence in which α_4 is an anionic amino acid; α_5 is any amino acid; α_6 is Ile, Val, Leu, Nle or Phe; α_7 is Pro, Hyp, 3,4-dehydroPro, thiazolidine-4-carboxylate, Sar, any N-methyl amino acid or D-Ala; α_8 is an anionic amino acid; α_9 is an anionic amino acid; α_{10} is a lipophilic amino acid selected from the group consisting of Tyr, Trp, Phe, Leu, Nle, Ile, Val, Cha, Pro; and α_{11} is any amino acid.

According to a preferred embodiment of this invention, ABEAM preferably comprises a sequence homologous to amino acids 57-64 of hirudin, i.e. α_4 is Glu; α_5 is Glu; α_6 is Ile; α_7 is Pro; α_8 is Glu; α_9 is Glu; α_{10} is Tyr, Tyr(SO_3H), Tyr(OSO_3H) or (3-,5-diiodoTyr), and α_{11} is Leu and Ω is preferably OH. It should be noted that native hirudin contains Try(OSO_3H) at position 63. However, carboxy terminal hirudin peptides which contain Tyr(SO_3H) are reported to have identical anticoagulant activity as those which contain the native Tyr(OSO_3H).

Other ABEAM components within the scope of this invention may comprise those portions of any molecule known to bind to the anion binding site of thrombin. These include amino acids 1675-1686 of Factor V, amino acids 272-285 of platelet glycoprotein Ib, amino acids 415-428 of thrombomodulin, amino acids 245-259 of prothrombin Fragment 2 and amino acids 30 to 44 of fibrinogen Aα chain. In addition, the ABEAM component may be selected from any of the hirudin peptide analogues

described by J.L.Krstenasky et al., "Development of MDL-28,050, A Small Stable Antithrombin Agent Based On A Functional Domain of the Leech Protein, Hirudin", Thromb. Haemostas., 63. pp. 208-14 (1990).

Less desirably, the ABEAM may comprise hirudin variants synthesised by chemical and/or biological means, such as, for example, chemically modified, amino acid substituted analogues as described in WO 92/01712.

Three exemplary ABEAM sequences (Ω=-OH) are shown below in Table B. Residue α_{12} is absent in preferred embodiments. The α^1 residue and optionally the α_1 residue may be absent if the ABEAM comprises a Hirudin sequence. The α^1 and preceding residues may be absent if the ABEAM comprises a thrombin platelet receptor sequence.

Table B

			α^1	α_1	α_2	α_3	α_4	α_5	α_6	α_7	α_8	α_9	α_{10}	α_{11}	α_{12}
r-Hirudin 53-65			D	G	D	F	E	E	I	P	E	E	Y	L	Q
Thrombin Platelet Receptor	N	P	N	D	K	Y	E	P	F	W	E	D	E	E	K
Fibrinogen Aα 30-42			D	S	D	W	P	F	A	S	D	E	D	W	N

Other possible but less preferred ABEAM sequences include DFEPIPL.

The Connector Moiety

The hirulogs of Maraganore et al (described in US 519404) contain a linker between the C-terminal carboxy residue of the CSDM and the N-terminal amino residue of the ABEAM. In the compounds of this invention, the CSDM and the ABEAM are interconnected by a connector bonded to the CSDM at its N-terminal amino residue or as a side chain of a CSDM residue. It has unexpectedly been found that this invention enables the provision of highly active thrombin inhibitors.

In preferred embodiments the connector is coupled to the N-terminal amino group of the CSDM. The amino group typically forms an amide bond with a carbonyl group of the ABEAM.

In other embodiments, the connector is coupled to the CSDM as (or through) a side chain thereof. Thus in some compounds the connector is bonded to a side chain group of a CSDM amino acid residue: this arrangement is convenient when the side chain has a functional group, for example amino or carboxyl, which can react with a complementary functionality on the connector, e.g. to form an amide or ester linkage. In other compounds, the connector is bonded to the α-carbon of a CSDM amino acid residue directly or through a functional group. In one class of compounds the connector, or a side chain linked thereto, replaces the α-hydrogen of a CSDM amino acid residue.

The connector when arranged as a side chain or, one may say, a side chain extension of the CSDM does not have to be coupled to an α-carbon of an amino acid residue but may, for example, be coupled to a constituent of a non-peptide bond between two amino acid residues.

It has been found that compounds of the invention, in which the connector does not form a C-terminal extension of the CSDM, are capable of adopting a configuration in which the ABEAM and CSDM can simultaneously bind to a thrombin molecule. Even more surprisingly, the invention enables provision of thrombin inhibitors having an improved Ki value over those of the exemplified hirulogs of WO 91/02750. The connector is selected to be capable of permitting simultaneous binding of the CSDM and the ABEAM to thrombin.

The connector preferably comprises a peptide "spacer" and a non-peptide "linker". A representative connector structure is:

wherein λ represents a non-peptide linker and σ a spacer comprising a sequence of amino acids, λ and σ suitably being joined by a peptide bond. The spacer σ is preferably linked to the ABEAM and the linker λ to the CSDM, although compounds in which σ is linked to the CSDM and λ to the ABEAM form a less preferred embodiment included in the invention.

The linker is typically a residue of a compound having functional groups to react with the N-terminal amino group of the spacer and a functional group of the CSDM, such as the N-terminal group, for example. A preferred linker, therefore, is a dicarboxylic acid which can form amide bonds with the N-terminal amino groups of the CSDM and the spacer. Particular preferred linkers are a residue of glutaric acid ($HO_2C(CH_2)_3CO_2H$ and homologues thereof of the formula $(HO_2C(CH_2)_hCO_2H$ wherein h is an integer of 2 or from 4 to 6. The alkylene residue [$-(CH_2)_{2-6}-$] may be substituted by one or more substituents which do not sterically hinder the linker, whereby the desirable flexibility of the linker is maintained..

Less preferably, the linker may comprise for example the residue of another compound having two carboxyl groups whose carbon atoms are separated by from 2 to 6 atoms.

The amino acid sequence of the spacer is not critical to the invention but it preferably comprises at least two adjacent Gly residues, normally at its N-terminal end. The length of the spacer is dependent upon *inter alia* the position on the CSDM to which the linker is attached. A particular problem which arises in specifying the length of the spacer is that of defining the junction between the ABEAM and the spacer. Thus, in compounds in which a glutaric acid linker is bonded to the P3 N-terminal amino group of a tripeptide (P3P2P1) CSDM, there is desirably a sequence of 5 amino acids between the glutaric acid residue and the Hir^{54} residue (Gly) of an ABEAM comprising Hirudin residues 53-56, but some of those 5 amino acids may be an extension of the Hirudin sequence. e.g. Hir^{51-53}.

Accordingly, one class of compounds is of the formula:

$$\begin{array}{l}\sigma\text{-Hir}^{54\text{-}64}\text{-}\Omega \\ \quad | \\ \text{OC-(CH}_2)_h\text{CO-(aa}^3\text{)-(aa}^2\text{)-(aa}^1\text{)-Z}\end{array}$$

wherein σ is a sequence of from 5 to 12 amino acids, preferably a sequence of from 5 to 10 amino acids and most preferably a sequence of from 5 to 7 amino acids. σ preferably includes at least 2 adjacent Gly residues an may include one or more consecutive Hir residues on the C-terminal side of Hir^{54}, e.g. $Hir^{53\text{-}51}$ or $Hir^{53\text{-}49}$; h is from 2 to 6 and is preferably 3; and the remaining symbols are as defined previously.

The moiety -σ-$OC(CH_2)_hCO$- has a length of from about 22Å (σ = 6 amino acid residues where h = 3) to 40Å (σ = 12 amino acid residues when h = 3), preferably of no more than about 34Å(σ = 10 amino acid residues when h = 3) and most preferably of no more than about 26Å (σ = 7 amino acid residues when h = 3). Alternative connector moieties having such lengths may be used.

The spacer (σ) may have one or more natural amide bonds replaced by other linkages, as described above in relation to the CSDM.

Variants

The essential feature of the inventive inhibitors is their possession of interlinked CSDMs and ABEAMs as discussed above. Other features of the compounds are not of the essence.

The compounds of the invention may therefore be in the form of a pharmaceutically acceptable salt thereof and/or comprise one or more protectable functional groups (e.g. -OH or $-NH_2$) protected by a pharmaceutically acceptable protecting group. Suitable salts include acid addition salts, as described above, and those of acid groups with Group I or Group II metal cations (e.g. Na^+, K^+, Mg^{2+}, Ca^{2+}). As protecting groups of

protectable functional groups. there may be mentioned t-butyl and benzyl as protecting groups for -OH and -COOH functions.

Affinity Properties

The inhibitory compounds of the invention have affinity for thrombin. Compounds which have affinity for an enzyme significantly inhibit or retard the enzyme's activity. It is desirable for the compounds to have an inhibition constant (Ki) for thrombin of 0.5 μM or less, preferably of 0.3 μM or less and most preferably of 0.1 μM or less. Particularly preferred compounds have a Ki of 0.05 μM or less is obtained. e.g. of 1nM or less, especially of less than 0.75nM. The Ki values herein refer to values determined at 37°C.

It is often preferred for the inhibiting compounds to be selective towards thrombin. In one class of inhibitory compounds, the ratio of Ki for non selected enzymes: Ki for thrombin is preferably at least 2 and more preferably at least 3. The Ki ratio may be at least 5.

A discussion of the inhibition constant Ki and a description of a method for determining it follows in the Examples.

Synthesis

The compounds of the invention can be prepared by using, for example, generally known methods for peptide synthesis and for coupling peptides. In an exemplary method, the novel compounds are made by a solid phase synthetic technique.

The invention therefore includes a method of making a compound of the invention, comprising the following steps:

(i) providing a solid phase having coupled thereto functional groups capable of reacting with an amino group or, preferably, with a carboxyl group or a reactive derivative thereof;

(ii) causing an amino or carboxyl group (which may be in the form of a reactive derivative thereof) of a terminal amino acid of an amino acid sequence of a compound of the invention selectively to react with said functional groups;

(iii) coupling the amino acid sequentially following, in the target sequence, the sequentially preceding amino acid coupled to the solid phase to said preceding amino acid; and

(iv) repeating step (iii) as often as necessary.

In step (i), the functional groups coupled to a solid phase may be on a moiety which is incorporated in the end product compound, e.g. may be an amino group (which may be derivatised) of an amino acid coupled directly or indirectly to the solid phase.

One or more additional steps may be, and normally are, included in the method in order to obtain the compound of the invention. Thus, preferred methods include, when desired, a step (v) of coupling a said sequentially following amino acid of a step (iii) to said preceding amino acid of the step through a compound having two functional groups capable of reacting with an amino group, whereby one of said functional groups becomes bonded to the amino group of said preceding amino acid and the other to the amino group of said following amino acid.

Any sequentially following amino acid of step (iii) may be part of a larger moiety, e.g. of an amino acid sequence optionally containing a replacement for a natural peptide bond.

In the method, any one or more carboxylate groups reacted with an amino group may be in the form of a reactive carbonyl-containing derivative thereof, such as an activated carboxyl group, for example and acid anhydride.

Before use the final compound of the solid phase synthesis is cleaved from the solid phase, for example in a manner known *per se*. The cleaved compound may be subjected to one or more further chemical reactions before the end product compound is obtained.

In a preferred embodiment, the terminal amino acid reacted with the functional groups attached to the solid phase is the C-terminal amino acid of the ABEAM and step (iii) is repeated to couple successive amino acids of the ABEAM sequence and successive amino acids of any contiguous connector peptide.

The final amino acid of the uninterrupted amino acid sequence thereby coupled to the solid phase is normally reacted with a compound having two carboxylate groups or reactive derivatives thereof, for example the anhydride of a dicarboxylic acid, to bond one of the two carboxylates to the amino group of the final amino acid. The unreacted carboxylate or carboxylate derivative is typically reacted with the amino group of an amino acid, which is normally the N-terminal amino acid of the CSDM. In this latter case, the amino acid may already be bonded to the remainder of the CSDM, i.e. the CSDM may be separately made in whole (or in part) for joining to the unreacted carboxylate (derivative). The compound having two carboxylate groups is preferably a linker as described above.

In preferred methods, there is used a preformed CSDM having a heteroatom group in place of a C-terminal carboxy group. The heteroatom group is preferably a boronate or boronate derivative as described above.

An amino acid or other moiety reacted with the solid phase material (the solid phase and any attached molecules) desirably has all its reactive functional groups protected other than the group to be reacted with the solid phase material. Any protected functional

group of the reacted amino acid or moiety which is subsequently itself to be reacted is deprotected before it is subjected to reaction.

A first preferred method, therefore, comprises:

(i) providing a solid phase having coupled thereto functional groups capable of reacting with a carboxyl group or a reactive derivative thereof;

(ii) contacting the solid phase with the C-terminal amino acid of an ABEAM, the amino acid having a protected amino group and optionally a derivatised carboxy group, and causing or allowing the carboxy groups of the amino acid molecules to react with the functional groups of the solid phase;

(iii) deprotecting the amino groups of the reacted amino acid, whereby the solid phase becomes provided with free amino groups;

(iv) repeating steps (ii) and (iii) with successive amino acids of the ABEAM and of a contiguous spacer peptide to form on the solid phase an amino acid sequence from the C-terminal of the ABEAM to, at the free end of the sequence, the N-terminal of the spacer;

(v) contacting the solid phase with a linker compound having two carboxyl groups or reactive residues thereof, and causing or allowing linker carboxy groups or reactive carboxy residues to react with the N-terminal amino groups of the spacer sequence;

(vi) contacting the solid phase having the linker compound coupled thereto with the N-terminal amino acid of a CSDM sequence and causing or allowing the amino groups of the amino acid molecules to react with the carboxy groups or reactive carboxy residues of the linker compound, the N-terminal amino acid of the CSDM sequence optionally being part of a complete CSDM;

(vii) if necessary, repeating steps (ii) and (iii) with successive amino acids of the CSDM to complete the CSDM sequence; and

(viii) cleaving the resultant compound from the functional groups of the solid phase.

A second preferred method comprises:

(i) providing a solid phase having coupled thereto functional groups capable of reacting with a carboxyl group or a reactive derivative thereof;

(ii) contacting the solid phase with the C-terminal amino acid of an ABEAM, the amino acid having a protected amino group and optionally a derivatised carboxy group, and causing or allowing the carboxy groups of the amino acid molecules to react with the functional groups of the solid phase;

(iii) deprotecting the amino group of the reacted amino acid, whereby the solid phase becomes provided with free amino groups;

(iv) repeating steps (ii) and (iii) with successive amino acids of the ABEAM to form on the solid phase an amino acid sequence from the C-terminal of the ABEAM to, at the free end of the sequence, the N-terminal of the ABEAM;

(v) contacting the solid phase with a linker compound having two carboxyl groups or reactive residues thereof, and causing or allowing linker carboxy residues to react with the N-terminal amino groups of the ABEAM;

(vi) contacting the solid phase having the linker compound coupled thereto with the N-terminal amino acid of the peptide spacer sequence and causing or allowing the amino groups of the amino acid molecules to react with the carboxy groups or reactive carboxy residues of the linker compound;

(vii) repeating steps (ii) and (iii) with successive amino acids of the spacer and then repeating step (ii) with the N-terminal amino acid of a CSDM sequence, the N-terminal amino acid of the CSDM sequence optionally being part of a complete CSDM;

(viii) if necessary, repeating steps (ii) and (iii) with successive amino acids of the CSDM to complete the CSDM sequence; and

(ix) cleaving the resultant compound from the functional groups of the solid phase.

In either of the preceding methods the synthesised compound is preferably cleaved from the solid phase by acid.

The preceding methods preferably involve the use of a CSDM amino acid or amino acid sequence (e.g. a complete CSDM) having a C-terminal boron group.

In the first and second preferred methods the functional groups coupled to a solid phase may be part of a moiety which is incorporated in the end product compound, e.g. may be an amino group (which may be derivatised) of an amino acid coupled directly or indirectly to the solid phase.

Solid phase synthesis is a technique familiar to peptide chemists and detailed elucidation is therefore not required here. An introduction to the technique may be found in "The Chemical Synthesis of Peptides", John Jones, Clarendon Press, Oxford, England, 1991. It may be mentioned, however, that in the first and second preferred methods described above, the amino groups of the amino acids used in step (ii) are suitably protected with Fmoc (fluorenylmethylcarbonyl), which is very labile in, and deprotectable by, secondary bases (e.g. piperidine). An alternative - and acid labile - protecting group is Boc (tertiarybutyloxycarbonyl). Trifluoracetic acid is typically used to cleave the Boc group between couplings.

In Fmoc chemistry (also known generally as the 'Sheppard approach'), the carboxy terminus of a peptide (or even the first amino acid) is coupled to a resin bead via a linker which is terminated by a 'reactive function'. The resin bead itself is typically polystyrene (PS), though other solids have been used that have suitable swelling characteristics in solvent, since it is now known that the peptide chain grows in the pores on the inside of the bead. An example of an alternative solid is the polyamide called Kiesulguhr.

The linker can be many things, but we prefer to use PEG (i.e. a polyethylene glycol linker), which terminates in an alcohol function.

The terminus of the linker, typically called a 'handle', depending on the desired product, but for Fmoc chemistry will be a moiety such that it can finally be cleaved by acid. The most common terminus (which we used) is HMBA or para-hydroxymethylbenzoic acid linker. The HMBA is esterified onto the PEG, and then the amino acid (with Fmoc on its N-terminus) is reacted to give also an ester link to the HMBA. The ester links are then cleavable by acid.

The resin used in Boc chemistry (also known more generally as the Merrifield method) is often divinylbenzyl based, for instance a 'Wang' resin has chloromethyl benzene co-polymerised to 2% divinylbenzene. The link to the resin is typically cleaved (very carefully!) by dry, liquid HF. This is described as 'vigorous' acidolysis.

The two classical methods of solid phase peptide synthesis (Sheppard and Merrifield), therefore, involve coupling amino acids via their carboxy-termini or their derivatives to a solid resin particle, then sequentially coupling new amino acids (via their activated carboxy termini) to via the N-termini generated.

Alternatively recent reports have shown coupling to the resin via the N-termini, for example via an acid labile benzyloxycarbonyl linkage, subsequent liberating of the carboxy termini, activating of these and coupling of amino acids via their N-termini,

the carboxy termini of the amino acids temporarily being protected. (Sharma, R.P.; Jones, D.A.; Broadbridge, R.J.; Corina, D.L. and Akhtar. M. A Novel Method of Solid Phase Synthesis Of Peptide Analogues, in Innovation and Perspectives in Solid Phase Synthesis, ed., R.Epton, 1994, Mayflower Worldwide Limited, Birmingham, page 353-356; Letsinger, R.L. and Kornet, M.J. J.Amer.Chem.Soc., 1963, 85, 3045.)

Such N-terminal coupling methods may be used in making the products of the invention. In one embodiment the CSDM, including any directly attached amino acid(s), is synthesised by N-terminal coupling. This technique is especially useful if the CSDM has C-terminal heteroatom group; in this method the resin bound peptide chain made using N-terminal coupling is derivatized to activate its carboxy termini, then a free α-aminoboronate ester or acid is coupled to the resin bound sequence. Finally the peptide boronate (comprising the CSDM) is cleaved from the resin by strong acid (e.g. HF or TFA) prior to being joined to the remainder of the final product.

When synthesising compounds whose CSDM contains a non-natural amide bond, it is convenient to premake as intermediates the binding subsite affinity moiety [X-$(aa^4)_m$-$(aa^3)_n$-(aa^2) of Formula I] and the specificity pocket affinity moiety with its attached C-terminal group [(aa^1)-Z of Formula I]. The two intermediates contain suitable functional groups to react together to form the target non-natural amide bond [ψ of Formula I] and are caused or allowed to react together to form the compound (or a precursor thereof to undergo one or more further functional group transformations).

Suitable synthetic techniques for making peptides containing non-amide bond ψ are described in PCT/GB96/00352. Suitable intermediates for such peptides are of the formula X-$(aa^4)_m$-$(aa^3)_n$-(aa^2)-G^1 or W-G^1 and G^2-(aa^1)-Z or G^2-A-Z, wherein G^1 and G^2 are groups which may be reacted together to form a linking group other than a natural amide bond, optionally after "working up" (e.g. hydrogenation) of the direct product.

Exemplary G^1 and G^2 groups are as follows:

Table B

ψ	G^1	G^2
$-CO_2-$	-COOH	leaving group, e.g. Cl. Br, I
$-CH_2O-$	$-CH_2OH$	Leaving group, e.g. Cl, Br, I
$-COCH_2-$	$-COCH_2Br$, or $-COCH_2Boc$	$\dot{C}HR-Z^*$ Leaving group, e.g. Cl. Br, I
-CH(CN)NH-	-CHO	$\dot{C}HR-Z^*$
$-CHOHCH_2-$	-CHO	CHR-Z*
-CH=CH-	$-NHCHRCH_2COH$	$(EtO)_2PO-$
$-CH_2-CH_2-$		make -CH=CH- then hydrogenate
$-CH_2NH-$	-CHO	H_2N-

In Table B the symbol "R" designates the side chain of the P1 amino acid.

* = G^2-(aa^1)-Z

Some representative syntheses of ψ linkages are described in more detail below:

ψ(CO_2) :

Species where (aa^2) has a free carboxyl group (CO_2H), and G^2 is a leaving group, preferably a halogen, and the base DBU (Diazabicycloundecane) are used.

ψ(CH_2O):

Species (aa^2) which contain a free hydroxyl group (OH), and G^2 is a leaving group, especially halogen, e.g. Cl, Br and the base DBU or an organolithium (e.g. Butyl lithium) are used.

$\psi(CH_2CH_2)$ where Z is $-P(O)(R^8)(R^9)$ or $-P(R^8)(R^9)$:

(aa_2) has a CH_2Hal G^1 group (Hal = halogen) and group G^2 is WCH_2Z, and a base such as NaH or BuLi is used.

$\psi(CH_2N)$:

Species (aa_2) which has an aldehyde (CHO) G^1 group and G^2 is an amino group, and the reagent sodium cyanoborohydride are used.

$\psi(COCH_2)$:

Ketomethylene bonds ($COCH_2$) can be prepared by reaction of a unit $X-(aa^4)_m-(aa^3)_n-(aa^2)$- carbonyldiimidazole and the lithium salt of tert-butyl acetate to give a beta-diketone $X-(aa^4)_m(aa^3)_n(aa^2)-COCH_2COOtBU$, and alkylation with NaH and a halomethylketone (Hoffman, R.V. and Kim, H.O., Tet.Lett.,1992, 33, 3597-3582) or α-haloboronate (e.g. $Hal-CHRBO_2Pin$) or α-halophosphonate and subsequent hydrolysis.

Alternatively reaction of $X-(aa^4)_m-(aa^3)_n-(aa^2)$ with diazomethane, then HBr gives the halomethylketone $X-(aa^4)_m-(aa^3)_n-(aa^2)-COCH_2Br$, this is then reacted with the carbanion of CH_2RBO_2Diol or $RCH_2P(O)(OR')_2$.

ψ [CH(CN)NH]:

Prepared by the method of Herranz, R., Suarez-Gea, M.L., Vinuesa, S., Garcia-Lopez, M.T. and Martinez, M., Tet.Let., 1991, 32, 7579-7582 or Suarez-Gea. M.L., Garcia-Lopez, M.T. and Herranz, R.. J.Org.Chem., 1994, 59, 3600-3603: reaction of X-$(aa^4)_m$-$(aa^3)_n$-(aa^2)-CHO with trimethylsilylcyanide, $ZnCl_2$ and $NH_2(aa^1)Z$.

ψ(CHOHCH$_2$):

By the methods of Boyd, S.A., Mantei, R.A., Hsiao, C.N. and Baker, W.R., J.Org.Chem., 1991, 56, 438-442 or Kano, S., Yokomatsu, T. and Shibuya, S.. Tet.Lett., 1991, 233-236: reaction of X-$(aa^4)_m$-$(aa^3)_n$-(aa^2)CHO with the carbanion of CH_2RBO_2 diol or $CH_2RP(O)(OR')_2$.

ψ(COCHF):

By reaction of an oxazolone with $(CHR{=}CFCO)_2O$, in a modification of the method as described by Hong, W., Dong, L., Cai, Z. and Titmas, R., Tet.Lett.1992, 33. 741-744. Then hydroboron, possibly in the presence of Palladium catalyst, of the X-$(aa^4)_m$-$(aa^3)_n$-(aa_2)COCFCHRBO$_2$Diol.

ψ(CH$_2$=CH) and ψ (CH$_2$CH$_2$):

Reaction of X-$(aa^4)_n(aa^3)_n$NHCHRCH$_2$CO-H (as NH_2CHRCH_2COH is β-alaninol) and $(EtO)_2PO$-CHR-BO$_2$Diol with base (e.g. NaH), in a modification of the method of Rodriguez. M., Heitz, A. and Martinez, J.. Int.J.pep.Prot.res., 1992. 39, 273-277. This gives the unsaturated analogue ψ(CH=CH) of the form X-$(aa^4)_m$-$(aa^3)_n$NHCHR^{1}CH2CH=CHRBO$_2$Diol. This can be hydrogenated with palladium on charcoal to give X-$(aa^4)_m$-$(aa^3)_n$NHCHR^1-$CH_2CH_2CH_2$-CHRBO$_2$Diol. ψ(CH=CH) could be prepared by the methods described by Ibuka, T., Yoshizawa, H., Habashita, H., Fuji, N.,Chounan, Y., Tanaka, M., and Yamamoto, Y., Tet.Lett., 1992, 33, 3783-3786 or Ibuka, T., Habashita, H., Otaka, A., Fuji, N., Oguchi, Y., Uyehara. T. and Yamamoto, Y., J.Org.Chem., 1991, 56, 4370-4382.

Other Dupont, V., Grand, V., Zerkout, S., Lecoq, A., Boussard, G., Vidal, J., Collet, A., and Aubry, A. "Modifications of the Amide Bond and Conformational Constraints in Pseudoamide Analogues", Biopolymers, 1993, 33, 1135-1148 or Gante, J. "Peptidomimetics-tailored enzyme Inhibitors". Angew.Chem.Int.Ed.Engl., 1994, 33, 1685-1698.

The reaction is preferably carried out in a dry, aprotic, polar solvent for example tetrahydrofuran, at a temperature between about -79°C and room temperature (typically, 20°C).

The intermediates may be obtained by the methods disclosed herein or alternatively by general methods as described in Matteson et al, Organometallics, 3, 1284-8 (1984), or as in Elgendy et al, Tet.Lett. 1992, 33, 4209-4212 or Tetrahedron 1994, 50, 3803-3812 or Rangaishenvi et al, J.Org.Chem 1991, 56, 3286-3294, or in EP-A-0599633. Suitable replaceable protecting groups may be used, for example as outlined for instance in Greene, T.W. and Wuts, P.G.M., "Protective Groups in Organic Chemistry", Wiley-Interscience, 1991. The starting amino acid(s) for the preparation of the protected peptide of intermediate may be prepared by standard, well-known methods such as those described for example in Angew. Chem. 93, 793 (1981), J.Am Chem. Soc., 109, 6881 (1987) and J Jones, "The Chemical Synthesis of Peptides", Oxford Science Publications, No. 23, Clarendon Press, Oxford 1992, or may be obtained from a variety of well known commercial sources.

Additionally or alternatively, a non-peptide bond may be incorporated during solid phase synthesis, for example using a method described in EP 257742 and equivalent US 4803261 (which is included herein by reference).

USE

The novel compounds according to the present invention are useful as inhibitors or substrates of thrombin, and may be used in vitro or in vivo for diagnostic and mechanistic studies of thrombin. More generally, the novel peptides may be useful for research or synthetic purposes. Furthermore, because of their inhibitory action, the inhibitors are useful in the prevention or treatment of diseases caused by an excess of thrombin in a regulatory system particularly a mammalian system, e.g. the human or animal body, for example control of the coagulation system. The pharmaceutically useful compounds have a pharmaceutically acceptable group as any N-terminal substituent (X).

The compounds of the invention may be employed when an anti-thrombogenic agent is needed. Generally, these compounds may be administered orally or parenterally to a host in an effective amount to obtain an anti-thrombogenic effect. In the case of larger mammals such as humans, the compounds may be administered alone or in combination with one or more pharmaceutical carriers or diluents at a dose of from 0.02 to 10mg/Kg of body weight and preferably 1-100mg/Kg, to obtain the anti-thrombogenic effect, and may be given as a single dose or in divided doses or as a sustained release formulation. When an extracorporeal blood loop is to be established for a patient, 0.1-10mg/Kg may be administered intravenously. For use with whole blood, from 1-100 mg per litre may be provided to prevent coagulation.

Pharmaceutical diluents or carriers for human or veterinary use are well known and include sugars, starches and water, and may be used to make acceptable formulations of pharmaceutical compositions (human or veterinary) containing one or more of the subject peptides in the required pharmaceutically appropriate or effective amount or concentration. The pharmaceutical formulations may be in unit dosage form. Formulations of the compounds include tablets, capsules, injectable solutions and the like.

The compounds of the invention may also be added to blood for the purpose of preventing coagulation of the blood in blood collecting or distribution containers, tubing or implantable apparatus which comes in contact with blood.

Advantages enabled by the invention include oral activity, rapid onset of activity and low toxicity. In addition, these compounds may have special utility in the treatment of individuals who are hypersensitive to compounds such as heparin or other known inhibitors of thrombin or other serine proteases.

The invention will be further described and illustrated by the Examples which now follow.

EXAMPLES

In the examples, amino acid residues are of L-configuration unless otherwise stated.

1. [-D-Phe-Pro-BoroBpgOPin] $CO(CH_2)_3COGly_2$-Gln(Tyr^{63})Hir^{51-64}

a. **GlyGlyGln(Tyr^{63})Hir^{51-64}**

GlyGlyGln(Tyr^{63})Hir^{51-64} which has the amino acid formula: H-Gly-Gly-Gln-His-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Tyr-Leu-OH, was prepared by solid phase peptide chemistry on a Milligen® 9050 PepSynthesizer using an Fmoc-polyamide continuous flow method and proprietary 9050 Plus on column monitoring software. Pre-derivatised solid support, Fmoc-Leu-PEG-PS (1.6g, 0.22meq/g) was used throughout; Fmoc-Leu-PEG-PS comprises polyethylene glycol derivatised polystyrene with HMBA linker. Fmoc groups were removed using 20% piperidine in DMF. Fmoc-amino acids (4 equiv.) as their pentafluorophenyl esters with side chain protection where appropriate (e.g. Fmoc-L-Tyr(tBu)OPfp, Fmoc-L-Glu(tBu)OPfp, Fmoc-L-Asp(tBu)OPfp, Fmoc-L-Asn(Trt)OPfp and Fmoc-His(boc)OPfp, were coupled sequentially. Once the required

peptide sequence was complete the N-terminal Fmoc group was removed using 20% piperidine in DMF. A positive ninhydrin test indicated that the Fmoc group had been removed. The peptide-conjugated resin was subsequently decanted on a filter and washed 'off line' with dichloromethane, methanol and dichloromethane before being dried *in-vacuo* for a few hours.

b. $HO_2C(CH_2)_3COGlyGlyGln(Tyr^{63})Hir^{51-64}$

The peptide obtained in Example 1a was suspended in DMF (5ml) and treated with glutaric anhydride (300mg) and 4-methyl-morpholine (200mg) in a round bottomed flask (25ml). The reaction mixture was swirled overnight. The resin was washed with DMF, DCM and MeOH. and then dried *in-vacuo* overnight to obtain the target compound.

c. H-D-Phe-ProBoroBpgOPin

H-D-Phe-ProBoroBpgOPin was prepared by adding a 40% solution of HBr in acetic acid (20ml) to Cbz-D-Phe-Pro-BoroBpgOPin (2g) in a round bottomed flask (100ml) fitted with a septum and flushed with nitrogen. The flask was swirled to effect complete dissolution of the protected tripeptide. When the gas evolution ceased after approximately 30 minutes, anhydrous ether (200ml) was added and the reaction mixture was stored in a refrigerator for 4 hours. The reaction mixture was filtered, the residue was dissolved in EtOH (1ml) and dry ether was added to precipitate the produce (800mg) as a white solid (M+H), 516; Tlc (C/M/A, 95/5/3), Rf=0.05.

d. $[\text{-D-PheProBoroBpgOPin}]CO(CH_2)_3COGly_2Gln(Tyr^{63})Hir^{51-64}$

To synthesise $[\text{-D-PheProBoroBpgOPin}]CO(CH_2)_3COGly_2Gln(Tyr^{63})Hir^{51-64}$, the dry resin $HOCO(CH_2)_3COGly_2Gln(Tyr^{63})Hir^{51-64}$ was suspended in DMF (10ml), before TBTU (129mg, 0.4mmol) and H-D-Phe-ProBoroBpgOPin (230mg, 0.4mmol) were

added to the reaction mixture. After 5 minutes stirring, triethylamine (40mg, 0.04mmol) was added and the flask left stirring overnight.

The fully protected peptide resin was washed with dichloromethane, methanol and dichloromethane and then dried under vacuum. Cleavage from the resin with simultaneous deprotection of side chain protecting groups was achieved by treating the resin with 100% TFA for two hours. TFA was removed and the free peptide with a C-terminal carboxylic acid was generated by precipitation with cold dry ether. The crude peptide was collected by filtration and washed with further portions of ether.

Purification of the crude peptide was carried out by reversed-phase HPLC using a Vydac™ C-18 preparative column (TP silica, 10μm, 25mm x 300mm). The column was eluted with a 30-90% linear gradient of solvent A (0.1% TFA in water) and solvent B (0.1 TFA in acetonitrile). The column eluants were monitored at 230nM, and fractions were collected appropriately. The purity of the products were determined by analytical RP-HPLC and mass spectrometry.

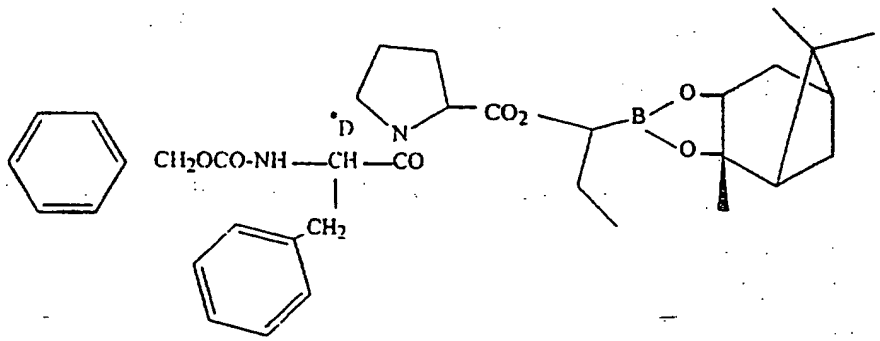
A schematic drawing of this compound, designated TRI 166, binding to thrombin is shown in the Figure.

2. Cbz-D-Phe-Pro-ψ(CO_2)-boroethylglycine pinanediol

a. Cbz-D-Phe-Pro-ψ(CO_2)-BoroEtg pinanediol

1-Chloroethane-pinanediol boronate ester (0.321g,1.25x10-3mol) added with stirring to Cbz-D-Phe-Pro-OH (0.6g, $1.52x10^{-3}$mol). When the addition had been completed, DBU (0.23g, 1.52mmol) in CH_2Cl_2 was added to the mixture and allowed to stir at room temperature, before being left to stir for an extended period at 4°C before workup. The opaque liquid was washed with HCl (0.1M, 2x50 ml), $NaHCO_3$ (1%, 50ml). The organic layer was dried by vigorous stirring over anhydrous $MgSO_4$, and filtered off, to

remove the desiccant. The filtrate was concentrated under reduced pressure on a rotary evaporator, to afford a thick, viscous residue. Preliminary examination by ^{1}H N.M.R. showed the required crude product. The crude sample was dissolved in a small amount of MeOH, applied to the sephadex® LH20 column, and then eluted with a pump using the same solvents. The elution profile was followed with the aid of a U.V. lamp (226nM) and recorder. The void volume, fraction 1-6, and a further bulk volume were collected. From the shape of the chromatogram, it was deemed that fractions 1-6 would be the most likely fractions in which the tripeptide may be found. The fractions were concentrated individually to afford clear slightly coloured viscous residues. One fraction containing the bulk of the material when placed under high vacuum was later afforded as a slightly crystalline product (0.269 yield of 35%). N.M.R., FABMS (Fast Atom Bombardment Mass Spectrometry) and C, H, N were very strong (good) indicators that the compound has been formed.



b. H-Phe-Pro-ψ(CO_2)-BoroEtg pinanediol

Cbz-D-Phe-Pro-ψ(CO_2)-BoroEtg pinanediol (from Example 2a) was dissolved in MeOH (30ml) and treated with 10% Pd/C, and purged with argon with stirring, the flask evacuated and pumped with H_2 with stirring for 5H. Ninhydrin staining indicated deprotected product on TLC. The solution was purged with argon for 10 min, filtered and concentrated under reduced pressure to afford a thick black oil, which was dissolved in $CHCl_3$, filtered and concentrated. ^{1}H N.M.R. of the crude product indicated no protected product. The residue from above was chromatographed on a Sephadex LH20

chromatography column. ^1H (60 MHz) N.M.R. showed that the isolated compound displayed many of the characteristics expected on the basis of the putative structure. 122mg of the free amino boronate ester was isolated.

3. [D-Phe-Pro-BoroIrgOPin]$OC(CH_2)_3COG_2NHir^{49-64}$(des-S)

To a solution of [D-Phe-Pro-BoroBrOPin]$OC(CH_2)_3COG_2NHir^{49-64}$ (des-S) (260mg, obtained from ~ 1.5g of resin) in ethanol (10ml) is added isothiourea (4 equivalents) and the reaction is stirred under argon for 6 days. The solution is concentrated, and dissolved in MeCN and applied to an HPLC column (30x300mm) and eluted by a gradient from 1-60% MeCN:

Fraction	Weight	Mass spectrum	Identification
3	90mg	2459	
4-5	75	2613	product

Fractions 4-5 gives an amino acid analysis consistent with the required product, [D-Phe-Pro-BoroIrgOPin]$OC(CH_2)_3COG_2NHIR^{49-64}$ (des-S) (mm 2614).

* * *

Analytical and Activity Data

The following Table 1 contains activity data relating to the invention. In the Table, the designation "Z" denotes benzoyloxycarbonyl and "NHir" refers to normal hirudin. "NHir49-64(des-S) refers to the amino acid sequence from amino acid 49 to amino acid 64 of normal hirudin in which the native $Tyr(OSO_3H)^{63}$ is replaced by Tyr.

The compounds listed in Table 1 were prepared by the same or analogous methods to the compounds of the preparation Examples 1 and 2 above or, in the case of intermediates, were obtained from sources.

The following techniques were employed for activity measurement:

Plasma thrombin time (TT)

A volume of 150µl of citrated normal human plasma and 20µl of buffer or sample were warmed at 37°C for 1 min. Coagulation was started by adding 150µl of freshly prepared bovine thrombin (5NIHu/ml saline) and the coagulation time was recorded on a coagulometer.

A phosphate buffer, pH7.8. containing 0.1% bovine serum albumine and 0.02% sodium azide was used. The samples were dissolved in DMSO and diluted with the buffer. When no inhibitor was used DMSO was added to the buffer to the same concentration as that used in the samples. The inhibitor concentrations were plotted against the thrombin times in a semilogarithmic graph from which the inhibitor concentration that caused a doubling (40 sec) of the thrombin time was determined.

Determination of Ki

The inhibition of human α-thrombin was determined by the inhibition of the enzyme catalysed hydrolysis of three different concentrations of the chromogenic substrate S-2238.

200µl of sample or buffer and 50µl of S-2238 were incubated at 37°C for 1 min and 50µl of human α-thrombin (0.25 NIHµ/ml) was added. The initial rate of inhibited and uninhibited reactions were recorded at 4.5nm. The increase in optical density was plotted according to the method of Lineweaver and Burke. The Km and apparent Km were determined and Ki was calculated using the relationship.

$$V = \frac{Vmax}{1 + \frac{Km}{[S]} \cdot \left(1 + \frac{[I]}{Ki}\right)}$$

The buffer used contained 0.1M sodium phosphate, 0.2M NaCl, 0.5% PEG and 0.02% sodium azide, adjusted to pH 7.5 with orthophosphoric acid.

The samples consist of the compound disclosed in DMSO.

The reader is referred to Dixon, M and Webb, E. C., "Enzymes", third edition, 1979, Academic Press, the disclosure of which is incorporated herein by reference, for a further description of the measurement of Ki.

Table 1

	Ki μM	TT μM
98 GGGDFEPIPL	n/e	100
99 [-BoroBrOPin]CO$(CH_2)_3$COGGGDFEPIPL	n/e	58
105 GGGGDFEPIPL	n/e	94.9
106 GGGGGDFEPIPL	n/e	15
107 [-BoroBrOPin]OC$(CH_2)_3$COGGGGDFEPIPL	n/e	58.4
108 [-BoroBrOPin]OC$(CH_2)_3$COGGGGGDFEPIPL	n/e	63.4
114 GGNSHNDGDFEEIPEEYL Hir^{49-64}	0.613	2
121 $HO_2C(CH_2)_3$COGGGGGDFEPIPL	0.738	30.9
128 [-L-PheProBoroValOPin]OC$(CH_2)_3COG_5$DFEPIPL	n/e 11.7	27.1
129 [-D-PheProBoroValOPin]OC$(CH_2)_3COG_5$DFEPIPL	16.4	33.5
137 [-D-Phe-ProBoroEtgOPin]OC$(CH_2)_3COG_5$DFEPIPL	1.23	10.2
166 [-D-Phe-ProBoroBpgOPin]OC$(CH_2)_3COG_2NHir^{49-64}$(des-S)	0.000649	0.029
167 Hir^{49-64}(des-S)	n/e 0.155	N.T.
175 [-D-Phe-ProBoroBpgOPin]OC$(CH_2)_3COG_4NHir^{49-64}$(des-S)	0.00211	0.065
176 Z-D-Phe-Pro-BoroBpgOPin + Hir^{49-64}	0.0218	0.636
182 [-D-Phe-ProBoroCegOPin]OC$(CH_2)_3$COGPGGNHir^{49-64}(des-S)	0.00271	N.T.
184 $HO_2C(CH_2)_3$COGPGGNHir^{49-64}(des-S)	12.7	56.7
185 [-D-Phe-ProBoroCegOPin]OC$(CH_2)_3COGPG_3NHir^{49-64}$(des-S)	n/e 0.75	7.36
186 [-D-Phe-Pro-BoroCegOPin]OC$(CH_2)_3COGPG_3NHir^{49-64}$(des-S)	n/e 0.9	4.61
267 [-PheProBoroCegPin]OC$(CH_2)_3COG_2$(EDFEPIPL)	0.762	4.9
268 [-$Pgl^D(OEt)_2$]OC$(CH_2)_3COG_2$(EDFEPIPL)	n/e 88.8	66.5
[-D-PheProBoroIrgOPin]OC$(CH_2)_3COG_2NHir^{49-64}$(des-S)	N.T.	N.T.

n/e = no effect

n/e 11.7 = no effect up to a concentration of 11.7μM

N.T. = not tested

CLAIMS

1. A thrombin inhibitor comprising

a) a catalytic site-directed moiety (CSDM) that binds to and inhibits the active site of thrombin;

b) an anion binding exosite associating moiety (ABEAM); and

c) a connector moiety bonded to the CSDM as an N-terminal extension or as or through a side chain thereof and to the ABEAM. the connector moiety being capable of permitting the CSDM and the ABEAM to bind simultaneously to a molecule of thrombin.

2. An inhibitor of claim 1, wherein the connector moiety is bonded to the CSDM as an N-terminal extension thereof.

3. An inhibitor of claim 1 or claim 2, wherein the connector comprises a peptide spacer portion and a non-peptide linker portion.

4. An inhibitor of claim 3, wherein the linker is a residue of a compound having functional groups to react with the N-terminal amino group of the spacer and a functional group of the CSDM.

5. An inhibitor of claim 4, wherein the linker has a carboxylic acid residue at opposed ends thereof.

6. An inhibitor of claim 5, wherein the linker is a residue of a dicarboxylic acid of the formula $HO_2C(CH_2)_hCO_2H$, wherein h is 2, 3, 4, 5 or 6.

7. An inhibitor of claim 6 wherein the linker is a glutaric acid residue.

8. An inhibitor of any of claims 3 to 7, wherein the spacer comprises at least two adjacent Gly residues.

9. An inhibitor of any of claims 1 to 8, wherein the CSDM is a residue of a compound of the formula

$$X\text{-}(aa^4)_m\text{-}(aa^3)_n\text{-}(aa^2)\text{-}(aa^1)\text{-}Z$$

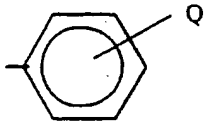
in which:

Z is a carboxyl or C-terminal extension group;

X is optionally replaced by the connector moiety and its attached ABEAM and, where present, is H (to form an H_2N- group) or an amino protecting group;

aa^1 is an amino acid residue of the formula -CH(J)-

wherein J is

$-C_qH_{2q}$-Q or C_qH_{2q} 

where Q is amino, amidino, imidazole, guanidino, N_3 or isothioureido and q is an integer of from 1 to 5,

or is a group of the formula

$$-(CH_2)_b\text{-}D_c\text{-}(CH_2)_d\text{-}E$$

wherein:

b and d are independently 0 or an integer such that (b+d) is from 0 to 4;

c is 0 or 1;

D is O; and

E is H, C_1-C_6 alkyl, C_1-C_6 haloalkyl, C_1-C_6 trialkylsilyl or C_6-C_{10} aryl optionally substituted by up to three groups selected from C_1-C_4 alkyl, halogen and C_1-C_4 alkoxy.

aa^2, aa^3 and aa^4 are residues of natural or unnatural amino acids or of amino acid analogues in which the α-hydrogen is replaced, at least one of the aa^2, aa^3 and aa^4 residues being hydrophobic,

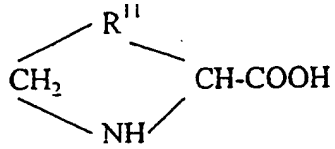
m is an integer from 0 to 7; and

n is 0 or 1.

10. An inhibitor of claim 9, in which n is 1.

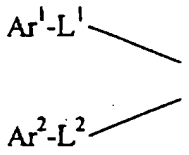
11. An inhibitor of claim 10, in which aa^2 and aa^3 are residues of amino acids with hydrophobic side chains.

12. An inhibitor of claim 11, in which aa^2 is the residue of an amino acid of the formula:



wherein R^{11} is $-CH_2-$, $-CH_2-CH_2-$, $-S-CH_2-$, $-S-C(CH_3)_2-$ or $-CH_2-CH_2-CH_2-$, which group is optionally substituted at one or more $-CH_2-$ groups by from 1 to 3 C_1-C_3 alkyl groups.

13. An inhibitor of claim 11 or claim 12, wherein n is 1 and aa^3 is the residue of a natural hydrophobic amino acid or is a residue of a group of the formula

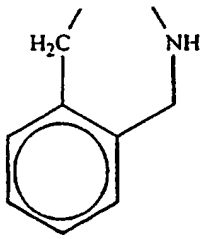


wherein

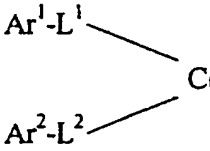
Ar^1 and Ar^2 are each independently selected from the group consisting of H; phenyl; phenyl substituted by halogen, a C_1-C_6 group which is alkyl or alkyl substituted or interrupted by a carbonyl or carbonyloxy group (e.g. alkylcarbonyl or alkoxycarbonyl) or substituted by $-R^{14}$ or $-OR^{14}$ wherein R^{14} is a 5- or 6-membered aromatic or non-aromatic ring or is C_1-C_4 alkyl substituted by such a 6-membered ring; bipyridyl; furanyl; chromanyl; quinolinyl; thienyl; pyridyl; α- or β-naphthyl; thionaphthyl; indolyl; p-iodophenylalanyl; diphenyl-methyl; fluorenyl; wholly or partially saturated groups corresponding to any of these; Me_3Si; or 2,2,2-trichloroethyl; any of the foregoing groups optionally being substituted by up to three groups selected from C_1-C_3 alkyl, C_1-C_3 alkoxy, or $R^{13a}CO-$ wherein R^{13a} is H, CH_3 or C_2H_5, or by a group of the formula O-, wherein R^1 is as hereinbefore defined, $R^{13a}OR^{1a}-$ or $R^{13a}COR^{1a}-$, wherein R^{1a} is $-CH_2-$, $-C_2H_4-$ or $-C_3H_6-$,

L_1 and L_2 are each independently selected from the group consisting of CH_2, CH_2-CH_2, O-CH_2, S-CH_2, and a bond, and

V is H, or -NHV and one of Ar^1-L^1 and Ar^2-L^2 together form a group of the formula



14. An inhibitor of claim 13, wherein n is 1 and aa^3 is a residue of a group of the formula



wherein

wherein V and Ar^1-L^1 and Ar^2-L^2 are as defined in claim 13.

15. A compound of claim 11, wherein aa^2 is the residue of proline, 2- or 3-thioproline or of pipecolic acid, n is 1 and aa^3 is a residue of D-Phe; D-Phe substituted at the phenyl 2-position (i) by a C_1-C_6 group which is alkyl or alkyl substituted or interrupted by a carbonyl or carbonyloxy group (e.g. is alkylcarbonyl or alkyloxycarbonyl) or (ii) by a 5 or 6 membered aryl group; D-Dpa; Dba; Pms; α- or β-Nal; TMSal; Chg; Phg; D-Tiq; or a para ether of D-Tyr.

16. An inhibitor of any of claims 1 to 15, wherein the CSDM comprises a peptide whose terminal carboxy group is replaced by a boron acid group or phosphorus acid group or a derivative thereof.

17. An inhibitor of any of claims 1 to 15, wherein the CSDM comprises a peptide whose terminal carboxy group is replaced by a group of the formula III:

$$-\text{Het}\,(O)_s(Y)_{t-2s} \qquad \text{III}$$

wherein

Het is a heteroatom;

s is 0, 1 or 2;

t is the valency of Het, t-2s being an integer of at least 1; and

each Y is independently hydrogen, halogen, hydroxy, substituted hydroxy, substituted thiol, amino or substituted amino, wherein two hydroxy groups, two thiol groups or an amino group are/is optionally substituted by a single divalent substituent.

18. An inhibitor of claim 17, wherein Het is boron or phosphorus.

19. An inhibitor of claim 17 or claim 18, wherein each Y is independently F or other halogen, $O\Sigma^1$ or $N\Sigma^1\Sigma^2$, wherein Σ^1 and Σ^2 are independently selected from H, hydrocarbyl and hydrocarbylcarbonyl, the hydrocarbyl groups optionally being substituted by one or more moieties selected from halogen, OH or alkoxy and/or containing an ether or ester linkage (-O- or -COO-), which groups contain up to 20 carbon atoms or wherein two Y groups taken together form the residue of a diol or a dithiol.

20. A compound of claim 19, wherein Het is boron and two Y groups taken together form the residue of pinacol or pinanediol.

21. An inhibitor of any of claims 1 to 20, wherein the ABEAM comprises a sequence of up to 15 amino acids of which at least 4 are anionic amino acids.

22. An inhibitor of claim 21, wherein the ABEAM comprises at least 5 anionic amino acids.

23. An inhibitor of any of claims 1 to 20, wherein the ABEAM comprises a sequence of up to 10 amino acids of which at least 3 are anionic.

24. An inhibitor of any of claims 21 to 23, wherein the ABEAM has anionic amino acids as residues 3 and 4 from its C-terminus.

25. An inhibitor of any of claims 21 to 23, wherein the ABEAM has anionic amino acids as residues 4 and 5 from its C-terminus.

26. An inhibitor of any of claims 1 to 20, wherein the ABEAM comprises a moiety of the following formula:

$$-\alpha_1-\alpha_2-\alpha_3-\alpha_4-\alpha_5-\alpha_6-\alpha_7-\alpha_8-\alpha_9-(\alpha_{10})_{w1}-(\alpha_{11})_{w2}-(\alpha_{12})_{w3}-\Omega$$

wherein each of α_1 to α_{12} is an amino acid residue, w1, w2 and w3 are each independently 1 or 0 and Ω is a carboxy terminal residue (attached to the terminal carbonyl group) and in which moiety α_4, α_8 and α_9 and two other amino acid residues are anionic.

27. An inhibitor of claim 26, wherein Ω is selected from hydroxy, C_1-C_6 alkoxy, amino, mono- or di- (C_1-C_4) alkyl substituted amino or benzylamino.

28. An inhibitor of claim 26 or claim 27, wherein said other anionic amino acid residues include α_1 or α_2.

29. An inhibitor of any of claims 26 to 28, wherein said moiety includes at least two pairs of adjacent anionic amino acids.

30. An inhibitor of any of claims 26 to 29, wherein the anionic amino acids are selected from Gly and Asp.

31. An inhibitor of claim 26, wherein α_4 is Glu; α_5 is Glu; α_6 is Ile; α_7 is Pro; α_8 is Glu; α_9 is Glu; α_{10} is Tyr, Tyr(SO_3H), Tyr(OSO_3H) or (3-,5-diiodoTyr), and α_{11} is Leu and Ω is preferably OH.

32. An inhibitor of any of claims 1 to 20, wherein the ABEAM comprises amino acids 1675-1686 of Factor V, amino acids 272-285 of platelet glycoprotein 1b, amino acids 415-428 of thrombomodulin, amino acids 245-259 of prothrombin Fragment 2 and amino acids 30 to 44 of fibrinogen Aα chain, or is a hirudin peptide analogue.

33. A compound of the formula

$$\sigma\text{-Hir}^{54\text{-}64}\text{-}\Omega$$
$$\overset{|}{OC}\text{-}(CH_2)_hCO\text{-}(aa^3)\text{-}(aa^2)\text{-}(aa^1)\text{-}Z$$

wherein:

σ is a sequence of from 5 to 12 amino acids,

n is from 2 to 6,

aa^3, aa^2, aa^1 and Z are as defined in claim 9,

Ω is as defined in claim 26 or claim 27, and

the moiety -σ-$OC(CH_2)_hCO$- has a length of from about 22Å (σ = 6 amino acid residues when h = 3) to 40Å (σ = 12 amino acid residues when h = 3).

34. A compound of claim 33, wherein h is 3 and σ comprises a sequence of 6 or 7 amino acids.

35. A compound of claim 33 or claim 34, wherein σ includes at least two adjacent Gly residues.

36. A compound of any of claims 33 to 35, which further includes the feature(s) recited in one or more of claims 11 to 15 and/or in which Z is a group as defined in one of claims 16 to 20.

37. A pharmaceutical formulation comprising an inhibitor of any of claims 1 to 32 or a compound of any of claims 33 to 36 formulated for use as a human or veterinary pharmaceutical.

38. A pharmaceutical composition comprising an inhibitor of any of claims 1 to 32 or a compound of any of claims 33 to 36 and a pharmaceutically acceptable diluent, excipient or carrier.

39. An inhibitor of any of claims 1 to 32 or a compound of any of claims 33 to 36 for use as a human or veterinary pharmaceutical.

40. The use in the manufacture of a medicament for inhibiting thrombin of an inhibitor of any of claims 1 to 32 or of a compound of any of claims 33 to 36.

41. The use of an inhibitor of any of claims 1 to 32 or a compound of any of claims 33 to 36 in a diagnostic or mechanistic study involving thrombin.

42. The use of an inhibitor of any of claims 1 to 32 or a compound of any of claims 33 to 36 which has affinity for a coagulation enzyme to prevent coagulation of extracorporeal blood.

43. A method of treating by therapy or prophylaxis a bodily disease or disorder capable of treatment by inhibition of thrombin, comprising administering, e.g. orally or parenterally, to a human or animal patient a therapeutically or prophylactically effective amount of an inhibitor of any of claims 1 to 32 or a compound of any of claims 33 to 36.

44. A method of making an inhibitor of any of claims 1 to 32 or a compound of any of claims 33 to 36 comprising performing the following steps to make a target amino acid sequence:

(i) providing a solid phase having coupled thereto functional groups capable of reacting with an amino group or, preferably, with a carboxyl group or a reactive derivative thereof;

(ii) causing an amino or carboxyl group of a terminal amino acid of the target amino acid sequence selectively to react with said functional groups, the carboxyl group optionally being in the form of a reactive derivative thereof;

(iii) coupling the amino acid sequentially following, in the target sequence, the sequentially preceding amino acid coupled to the solid phase to said preceding amino acid; and

(iv) repeating step (iii) as often as necessary.

45. A method of claim 44 which further includes a step (v) of coupling a said sequentially following amino acid of a step (iii) to said preceding amino acid of the step through a compound having two functional groups capable of reacting with an amino group, whereby one of said functional groups becomes bonded to the amino group of said preceding amino acid and the other to the amino group of said following amino acid.

46. A method of claim 44 or claim 45, wherein the terminal amino acid reacted with the functional groups attached to the solid phase is the C-terminal amino acid of the ABEAM (Hir^{54-64} in the case of claim 33) and step (iii) is repeated to couple successive amino acids of the ABEAM sequence and successive amino acids of any contiguous connector peptide (σ in the case of claim 33).

47. A method of any of claims 44 to 46, wherein the final amino acid of the uninterrupted amino acid sequence coupled to the solid phase is reacted with a compound having two carboxylate groups or reactive derivatives thereof, and the unreacted carboxylate or carboxylate derivative is typically reacted with the amino

group of an amino acid which is the N-terminal amino acid of the CSDM ((aa^3)-(aa^2)-(aa^1)-Z in the case of claim 33).

48. A method of claim 47, wherein said N-terminal amino acid is already bonded to the remainder of the CSDM ((aa^3)-(aa^2)-(aa^1)-Z in the case of claim 33) and the CSDM has a heteroatom group in place of a C-terminal carboxy group (i.e. Z is a heteroatom group in the case of claim 33).

49. The method of any of claims 44 to 48 in which any one or more carboxylate groups reacted with an amino acid is in the form of a reactive carbonyl-containing derivative thereof.

50. A method of making an inhibitor of any of claims 1 to 32, the inhibitor having a connector as defined in claim 3 and optionally being of a formula defined in any of claims 33 to 36, comprising performing the following steps:

(i) providing a solid phase having coupled thereto functional groups capable of reacting with a carboxyl group or a reactive derivative thereof;

(ii) contacting the solid phase with the C-terminal amino acid of an ABEAM, the amino acid having a protected amino group and optionally a derivatised carboxy group, and causing or allowing the carboxy groups of the amino acid molecules to react with the functional groups of the solid phase;

(iii) deprotecting the amino groups of the reacted amino acid, whereby the solid phase becomes provided with free amino groups;

(iv) repeating steps (ii) and (iii) with successive amino acids of the ABEAM and of a contiguous spacer peptide to form on the solid phase an amino acid sequence from the C-terminal of the ABEAM to, at the free end of the sequence, the N-terminal of the spacer;

(v) contacting the solid phase with a linker compound having two carboxyl groups or reactive residues thereof, and causing or allowing linker carboxy groups or reactive carboxy residues to react with the N-terminal amino groups of the spacer sequence;

(vi) contacting the solid phase having the linker compound coupled thereto with the N-terminal amino acid of a CSDM sequence and causing or allowing the amino groups of the amino acid molecules to react with the carboxy groups or reactive carboxy residues of the linker compound, the N-terminal amino acid of the CSDM sequence optionally being part of a complete CSDM;

(vii) if necessary, repeating steps (ii) and (iii) with successive amino acids of the CSDM to complete the CSDM sequence; and

(viii) cleaving the resultant compound from the functional groups of the solid phase.

51. A method of making an inhibitor of any of claims 1 to 32, the inhibitor having a connector as defined in claim 3 and optionally being of a formula defined in any of claims 33 to 36, comprising performing the following steps:

(i) providing a solid phase having coupled thereto functional groups capable of reacting with a carboxyl group or a reactive derivative thereof;

(ii) contacting the solid phase with the C-terminal amino acid of an ABEAM, the amino acid having a protected amino group and optionally a derivatised carboxy group, and causing or allowing the carboxy groups of the amino acid molecules to react with the functional groups of the solid phase;

(iii) deprotecting the amino group of the reacted amino acid, whereby the solid phase becomes provided with free amino groups;

(iv) repeating steps (ii) and (iii) with successive amino acids of the ABEAM to form on the solid phase an amino acid sequence from the C-terminal of the ABEAM to, at the free end of the sequence, the N-terminal of the ABEAM;

(v) contacting the solid phase with a linker compound having two carboxyl groups or reactive residues thereof, and causing or allowing linker carboxy residues to react with the N-terminal amino groups of the ABEAM;

(vi) contacting the solid phase having the linker compound coupled thereto with the N-terminal amino acid of the peptide spacer sequence and causing or allowing the amino groups of the amino acid molecules to react with the carboxy groups or reactive carboxy residues of the linker compound:

(vii) repeating steps (ii) and (iii) with successive amino acids of the spacer and then repeating step (ii) with the N-terminal amino acid of a CSDM sequence, the N-terminal amino acid of the CSDM sequence optionally being part of a complete CSDM;

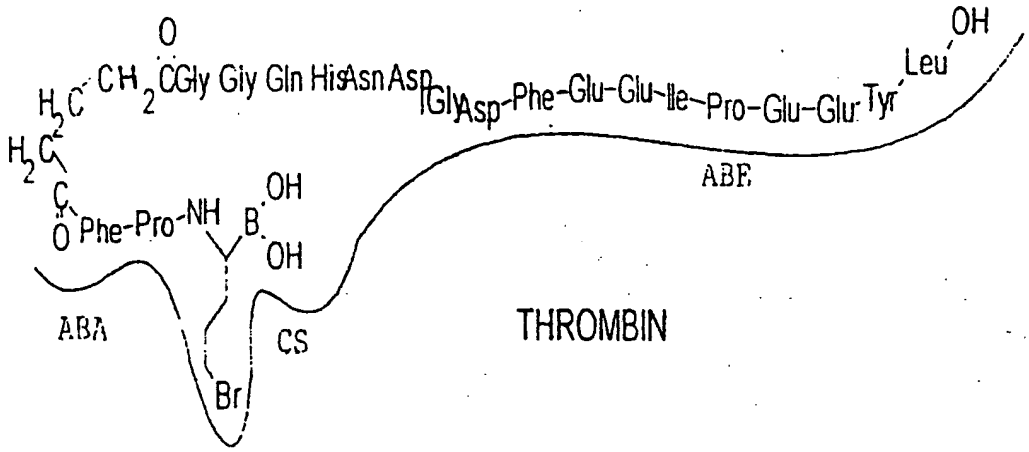
(viii) if necessary, repeating steps (ii) and (iii) with successive amino acids of the CSDM to complete the CSDM sequence: and

(ix) cleaving the resultant compound from the functional groups of the solid phase.

52. A method of claim 50 or claim 51, wherein the synthesised compound is cleaved from the solid phase by acid hydrolysis.

53. A method of any of claims 44, 45, 50, 51 or 52, in which the functional groups coupled to the solid phase are on a moiety which is incorporated in the end product compound.

54. A method of claim 53, in which said functional groups are derivatised amino groups of amino acids coupled directly or indirectly to the solid phase.



Key

ABA	=	apolar binding area
CS	=	catalytic site
ABE	=	anion binding exosite

INTERNATIONAL SEARCH REPORT

national Application No
PCT/GB 97/01575

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07K14/815 C07K5/065 A61K38/55 A61K38/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	S. ELGENDY ET AL.: "Design of a novel class of bifunctional thrombin inhibitors, synthesised by the first application of peptide boronates in solid phase chemistry" TETRAHEDRON LETTERS, vol. 38, no. 18, 5 May 1997, OXFORD GB, pages 3305-3308, XP002043733 see the whole document ---	1-54
X	WO 91 02750 A (BIOGEN INC ;HEALTH RESEARCH INC (US)) 7 March 1991 see claims; examples --- -/--	1-12,15, 21-32, 37-54

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

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Date of the actual completion of the international search	Date of mailing of the international search report
16 October 1997	29. 10. 97

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 97/01575

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92 13952 A (BIOGEN INC) 20 August 1992 see claims; examples	1-12,15, 21-32, 37-54
X	DIMAIO J ET AL: "A NEW CLASS OF POTENT THROMBIN INHIBITORS THAT INCORPORATES A SCISSILE PSEUDOPEPTIDE BOND" FEBS LETTERS, vol. 282, no. 1, 22 April 1991, pages 47-52, XP000147169 see page 48, left-hand column, paragraph 3 - right-hand column, last line; figure 1	1-12,15, 21-32, 37-54
P,X	WO 96 30407 A (CANADA NAT RES COUNCIL ;DIMAIO JOHN (CA); TARAZI MICHELINE (CA)) 3 October 1996 see claims; examples	1-12,15, 21-32, 37-54
P,X	WO 96 29347 A (CANADA NAT RES COUNCIL ;DIMAIO JOHN (CA)) 26 September 1996 see claims; examples	1-12,15, 21-32, 37-54
X	J. DIMAIO ET AL.: "Synthesis of a Homologous Series of Ketomethylene Arginyl Pseudopeptides and Application to Low Molecular Weight Hirudin-like Thrombin Inhibitors" JOURNAL OF MEDICINAL CHEMISTRY, vol. 35, no. 18, 4 September 1992, WASHINGTON US, pages 3331-3341, XP002043788 see Schemes I-III see page 3335, right-hand column, paragraph 1 - page 3337, left-hand column, paragraph 3; figure 1	1-12,15, 21-32, 37-54

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB 97/01575

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1 [X] Claims Nos.. 43
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 43 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3 [ ] Claims Nos
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/GB 97/01575

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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